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INHERITANCE OF RESISTANCE TO THE COMMON MOSAIC VIRUS IN THE BEAN[1]

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INTRODUCTION

The mosaic virus of the common bean (*Phaseolus vulgaris* L.) is coextensive with bean culture in the United States and Canada. Reports of the disease in other countries appear in the literature, and the work of Rands and Brotherton (*19*)[3] with varieties of beans from all parts of the world suggests that the virus is cosmopolitan in its distribution.

Owing to the fact that disease-free seed is difficult to produce and that elimination of the virus from infected seed is not possible, the only means of control available at present is the use of resistant or immune varieties. Michigan Robust is the only commercial variety of field beans that is immune. However, immune selections of the Great Northern variety of field bean (*17*) and the Stringless Green Refugee canning bean (*18*) are being released by the Idaho Agricultural Experiment Station. Two new mosaic-resistant snap beans of the Stringless Refugee type have been released under the designations of U. S. No. 1 and U. S. No. 5 by the United States Department of Agriculture since this investigation was started. Corbett Refugee, a selection from commercial Stringless Green Refugee, was the only immune canning-type bean available for the present investigation.

Several investigators have classified bean varieties according to their degree of resistance. Pierce and Walker, as reported by Clark (*3*), for example, group the varieties tested into three classes. Class 1 includes the varieties (Stringless Green Refugee, Refugee Wax, Red Valentine, etc.) that are susceptible to the common or Refugee-type virus and develop pronounced symptoms when infected; in class 2 are placed the tolerant varieties (Improved Kidney Wax, Full Measure, Giant Stringless, etc.), which are readily infected but which ordinarily do not develop marked symptoms; and in class 3 are grouped the resistant or immune varieties. In this paper are reported the results of crossing bean varieties belonging to the extremes (classes 1 and 3) in the above grouping in order to determine the inheritance of resistance to the common bean mosaic virus. In the early stages of the breeding work it was observed that resistance did not conform to any simple law of inheritance, and for that reason the program of hybridization was limited to a few varieties with a view to studying them in considerable detail.

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[2] The writer wishes to express his appreciation for the helpful assistance given during the course of this investigation by Dr. R. A. Brink and for the advice given by Dr. J. C. Walker, of the College of Agriculture, University of Wisconsin.

[3] Reference is made by number (italic) to Literature Cited, p. 914.

REVIEW OF LITERATURE

Since Iwanowski (*7*), in 1899, first mentioned the occurrence of a virus disease on beans, numerous observations have been published dealing largely with symptoms, environmental relations, transmission, distribution, and economic losses caused by the disease. Only one paper deals to any extent with the subject with which we are concerned, namely, the inheritance of resistance to bean mosaic.

McRostie (*9*), in 1921, reported the results of crossing resistant Michigan Robust with Flat Marrow and with a strain designated as Selection B. According to his data, McRostie obtained a high degree of resistance in the F_1 generation in certain cases. If one may assume that the first variety he lists in each case is the maternal parent, then it would appear that in crosses between resistant and susceptible varieties the F_1 was more resistant when the resistant variety was used as the female parent than as the male parent. The following is a summary of table 3 of McRostie's paper, showing the reactions of a series of F_1 individuals:

Robust × Flat Marrow, *b*, *b*, *b*, *b*, *b*, *b*.[4]
Flat Marrow × Robust, *e*, *d*, *d*, *d*, *b*, *b*.
Robust × Selection B, *d*, *d*.
Selection B × Robust, *b*, *d*.
Well's Red Kidney × Robust, *b*.

The F_1 plants from the Robust × Flat Marrow cross, as listed in McRostie's table, were all highly resistant (class *b*), while four out of six of the plants from the reciprocal cross were more or less severely infected. McRostie makes no mention of the apparent difference in the reciprocal crosses. However, this difference is not evident in the F_1 generation of Robust × Selection B and reciprocal. Both plants from the cross Robust × Selection B were heavily infected. One plant was highly resistant and one very susceptible in the cross Selection B × Robust. McRostie indicates that the results show susceptibility to be dominant, or partially so, over resistance. In the F_2 generation a ratio approximating nine susceptible to seven resistant was obtained when all F_2 results were grouped together without reference to the varieties used or the direction in which the crosses were made. Variations in degree of resistance and susceptibility in the F_2 from different crosses are suggested in the F_2 table, but McRostie attributes these to the small numbers involved. He states (*9*, *p. 22*):

> In view of the ratio reported, we must assume that two factors are concerned in producing susceptibility or resistance to this disease. The presence of both of these factors in the dominant condition goes to make susceptibility. The presence, on the other hand, of one or both of their recessive allelomorphs in the homozygous condition tends to produce a plant that is resistant to the disease in question.

McRostie was handicapped by the lack of a technique that would insure infection of all susceptible plants. In the greenhouse he used a rubbing method of inoculation, but in the field natural dissemination of the virus was relied upon, every third row being planted with seed from diseased plants.

Pierce and Walker (*18*), crossing Corbett Refugee and Stringless Green Refugee, found the F_1 hybrids to be resistant to mosaic and succeeded in isolating resistant progenies from resistant F_2 plants.

[4] The letters *a* to *e* indicate varying degrees of infection, *a* indicating no infection and *e* severe infection.

In the two investigations just reviewed two different ideas are expressed. McRostie reported resistance to be recessive, while Pierce and Walker found resistance to be dominant to susceptibility.

METHODS AND MATERIALS

The Stringless Green Refugee variety, a prolific green snap bean of high quality, which is extensively grown for canning, was used as the susceptible parent throughout the present investigation. It is very susceptible to mosaic, and the disease is an important agent in reducing the yields. Michigan Robust (Michigan No. 40520), a white pea bean, was used as a resistant parent. This strain is immune to the mosaic virus employed in these studies. Corbett Refugee, an immune selection from the Stringless Green Refugee variety, was also used as a resistant parent.[5]

Hybridizations between disease-free parent plants were made in the greenhouse, care being taken to avoid self or otherwise uncontrolled pollination. The flowers were emasculated when the edges of the standard petal of the corolla were just beginning to separate. Buds manipulated earlier were usually shed, and buds almost fully open were found to be self-pollinated.

The flowers were pollinated immediately after emasculation, tagged, and wrapped in paper or cotton to guard against foreign pollen and to prevent destructive drying of the exposed floral parts. The immunity of the resistant parent lines was established by inoculating representative samples with the mosaic virus. A few flowers of each plant used as a parent in crosses were self-pollinated and the reaction of the offspring to the virus was determined. All descendants from "selfed" resistant parents were immune. Likewise, all plants produced from self-pollinated flowers of susceptible parents were susceptible. This procedure gives conclusive evidence that plants used as resistant parents were immune and upon "selfing" transmitted the immunity to all of their offspring. It also proves that plants used as susceptible parents were "true" susceptibles.

The hybrid offspring and the parent strains were grown in a cheesecloth cage out of doors. With this method no difficulty was experienced in keeping the plants healthy. The general procedure in handling hybrid plants was as follows: A few F_1 individuals were inoculated with the virus in order to obtain an index of their reaction to the disease. The rest of the F_1 plants were kept healthy by growing them in the cheesecloth cage mentioned above; F_2 seed harvested from these plants, therefore, was disease-free. The F_2 plants were first exposed to the virus as seedlings. These inoculated plants were selfed and harvested separately. The F_3 generation, therefore, was derived from F_2 individuals whose reaction to the virus had been established. There was tested also an F_3 generation from plants that had been kept disease-free in the F_1 and F_2.

A portion of the seed from F_2 plants classified as resistant was planted and observed in the greenhouse in order to establish the fact that these plants were actually immune and were not carrying the virus in a masked condition. No diseased plants occurred among the offspring of such tested plants.

[5] Seed of the Corbett Refugee strain and disease-free seed of the Stringless Green Refugee variety were obtained from W. J. Zaumeyer, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Owing to the "hard-shell" character of the hybrid seed it was found necessary to scarify all seeds before planting, in order to obtain uniform germination. Newly harvested seed, if scarified, will germinate immediately upon being planted.

A leaf-rubbing method of inoculation was used, which consisted of macerating leaves from diseased plants in a mortar and expressing the juice through cheesecloth. The fully expanded primary leaves of the plants to be inoculated were rubbed on the upper surface along the midrib with a small piece of cheesecloth dipped in the infective juice. A small quantity of quartz sand was added as an abrasive agent. Fajardo (*6*) reported from 80- to 100-percent infection with this method. During the course of the present investigation no difficulty was encountered in producing 100-percent infection in plants known to be susceptible if a second inoculation, into the first compound leaf, was given a few days after the first. Figure 1 is a photograph

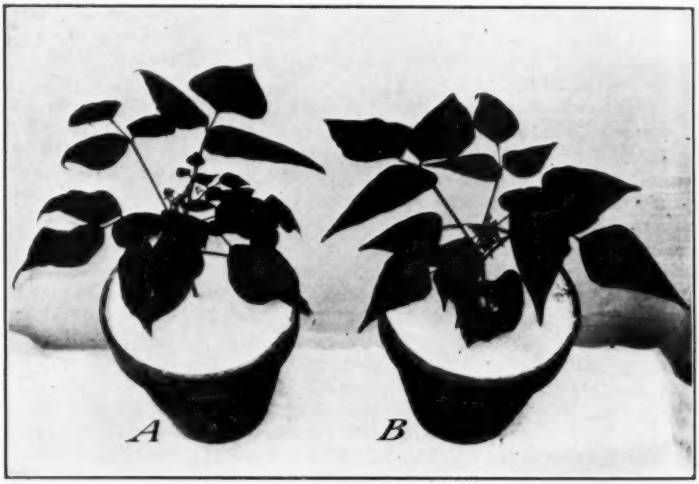

FIGURE 1.—F_2 bean plants 30 days after inoculation: *A*, Susceptible plant showing mosaic symptoms; *B*, resistant plant. The primary leaves show inoculation injury.

showing (*A*) susceptible and (*B*) resistant plants 30 days after inoculation. Evidence of the inoculation injury may be seen on the primary leaves.

The virus inoculum was obtained from the descendants of a single plant of the Stringless Green Refugee variety grown in the United States Department of Agriculture bean trials at Greeley, Colo., in 1931. The plant exhibited mosaic symptoms similar to other diseased plants in the vicinity and was selected as representative of the ordinary or common type of bean mosaic. It is the writer's opinion that this virus is identical with bean virus no. 1 as reported by Pierce (*16*). Disease-free Refugee plants were inoculated with virus extract from diseased offspring of this plant. The culture has been maintained by repeated inoculations of disease-free seedlings. This material has been the sole source of infective tissue for testing the reaction of parents and hybrid progenies to the disease. The plan was adopted

to avoid any complication that might result if different strains of the bean mosaic virus exist.

Disease-free seedlings of the Stringless Green Refugee variety were used as checks throughout the investigation. The infectivity of each extracted sample of inoculum used was established by observing the symptoms produced on plants of this stock. Every fifth row in greenhouse plantings was a check. In the field the check rows, containing approximately 30 plants each, were located at random. All susceptible check plants became infected following inoculation. This should be sufficient check on the technique employed and upon the environmental conditions prevailing to establish with certainty the fact that all susceptible plants in the segregating progenies were infected with the virus. The checks were included in all plantings, and in each case 100-percent infection was obtained by means of two inoculations as mentioned above.

Michigan Robust and Corbett Refugee were used as resistant checks. In no case did resistant check plants become infected. The beans were planted in the genetics plot on the University of Wisconsin campus at Madison, Wis., and were more or less isolated from other beans each year.

RESULTS

BEHAVIOR OF F_1 GENERATION OF MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE AND RECIPROCAL

In the F_1 generation of the Michigan Robust × Stringless Green Refugee combinations the reciprocal crosses were markedly unlike. The results of inoculating F_1 individuals of the cross susceptible ♀ × resistant ♂ [6] are presented in table 1.

TABLE 1.—*Reaction of F_1 generation of susceptible (Stringless Green Refugee) ♀ × resistant (Michigan Robust) ♂ and reciprocal to common bean mosaic*

STRINGLESS GREEN REFUGEE × MICHIGAN ROBUST

Parent no.	Plant no.	F_1 plants			Parent no.	Plant no.	F_1 plants		
		Tested	Healthy	Diseased			Tested	Healthy	Diseased
		Number	*Number*	*Number*			*Number*	*Number*	*Number*
1-2×1-16	34-3	1	0	1	3-1×3-31	101	1	0	1
1-2×1-17	35-2	1	0	1	3-2×3-21	102	3	0	3
1-2×1-22	36-4	1	0	1	3-3×3-20	103	2	0	2
1-3×1-11	37-3	1	0	1	3-3×3-28	104	1	0	1
1-5×1-12	38-3	1	0	1	3-3×3-24	105	2	0	2
1-8×1-12	39-3	1	0	1	3-4×3-26	106	2	0	2
1-10×1-12	40-5	1	0	1					
1-10×1-15	41-3	1	0	1	Total		21	0	21
3-1×3-20	100	2	0	2					

MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE

1-11×1-8	44-5	1	1	0	3-21×3-4	107	1	1	0
1-16×1-2	45-2, 3	2	1	1	3-22×3-1	108	2	1	1
1-20×1-99	46-5	1	1	0	3-24×3-3	109	2	2	0
1-23×1-96	47-4	1	1	0	3-27×3-2	110	1	1	0
1-17×1-2	49-1, 2	2	1	1					
1-20×1-88	50-1, 2	2	2	0	Total		17	14	3
1-22×1-2	51-1, 2	2	2	0					

[6] To avoid confusion in presenting and discussing these results, the maternal parent has been listed first in each case.

Twenty-one plants were tested, all of which proved to be susceptible. The results of inoculating F_1 individuals from the reciprocal cross are given in table 1. Only 3 out of 17 plants tested were susceptible to mosaic. These results might suggest that the F_1 plants were not hybrids but accidental selfs, since their reactions to the mosaic virus are similar to the reactions of the respective female parents. The Michigan Robust variety has white seeds, white flowers, and an indeterminate type of growth, whereas Stringless Green Refugee has purple-mottled seeds, pink blossoms, and a determinate type of growth. It was possible, therefore, to establish definitely the hybridity of the F_1 plants by the characters shown in this generation and by segregation in the F_2 generation.

The different behavior of reciprocal hybrids in relation to the mosaic virus must be explained in some other manner. The F_1 plants tend strongly to resemble the maternal parent with respect to susceptibility. If the female parent is susceptible to mosaic the offspring are susceptible, and if a resistant individual is used as a mother plant most of the offspring are resistant. These results are contrary to the Mendelian type of inheritance, where except in cases of sex linkage both parents contribute equally to the offspring.

In considering the additional data given below, a brief account of the writer's (*15*) results on the inheritance of a variegated leaf character in the bean will be helpful. Hybrids were made between plants with variegated leaves and normal green plants. All F_1 plants from the variegated ♀ × green ♂ cross were variegated. Seven out of eight F_1 plants from the reciprocal cross (normal green ♀ × variegated ♂) were normal green; one plant had a slight variegation on one leaf. This difference in reciprocals was again evident in the F_2 generation. In the second generation of variegated ♀ × green ♂, 84 percent variegated and 16 percent green plants were obtained. In the reciprocal cross (green ♀ × variegated ♂) 93 percent green and 7 percent variegated plants were found. These results suggest that the character of the F_1 and F_2 offspring is largely governed by the maternal parent and that the variegated condition is governed by the cytoplasm. The occurrence of some variegated offspring when the variegated plants are used as paternal parents suggests that the causal agency is introduced, to some extent, with the pollen tube at the time of fertilization.

Further discussion and explanation of the variegated character and its relation to mosaic resistance will be found in the section entitled "Discussion."

BEHAVIOR OF F_2 GENERATION OF MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE AND RECIPROCAL

The F_2 individuals were obtained from plants that were not inoculated in the F_1 generation. Recent workers have reported the transmission of mosaic by the pollen from diseased plants. Reddick (*20*) first reported this for beans in 1931. Nelson and Down (*10*) concluded that one-fourth of the ovules and approximately one-fourth of the pollen grains of diseased plants are infected and are instrumental in transmitting the disease to the next generation. Since it was desirable to avoid any complications that might arise from infection of some of the young embryos borne on susceptible mother plants or from infection of ovules of resistant mother plants

by diseased pollen from susceptible individuals, the plants that gave rise to the F_2 progenies were grown in a cheesecloth cage to keep them disease-free.

The striking difference in behavior of the reciprocal crosses shown in the F_1 is again apparent in the F_2 generation. The F_2 results of the susceptible × resistant cross are shown in table 2. Out of a total of 4,728 plants inoculated with the mosaic virus, only 60 plants, or 1.27 percent, remained healthy. On the other hand, 4,668 plants, or 98.73 percent, became infected.

TABLE 2.—*Reaction of F_2 generation of susceptible (Stringless Green Refugee) ♀ × resistant (Michigan Robust) ♂ and reciprocal to common bean mosaic*

STRINGLESS GREEN REFUGEE × MICHIGAN ROBUST

Progeny no.	F_2 plants					Progeny no.	F_2 plants				
	Number			Percent			Number			Percent	
	Tested	Healthy	Diseased	Healthy	Diseased		Tested	Healthy	Diseased	Healthy	Diseased
34–1	321	1	320	0.31	99.69	39–1	464	3	461	0.65	99.35
34–2	307	2	305	.65	99.35	39–2	497	5	492	1.01	98.99
35–1	426	6	420	1.41	98.59	40–1	41	0	41	.00	100.00
36–1	253	3	250	1.19	98.81	40–2	130	0	130	.00	100.00
36–2	212	8	204	3.77	96.23	40–3	403	1	402	.25	99.75
36–3	232	6	226	2.59	97.41	40–4	27	0	27	.00	100.00
37–1	268	3	265	1.12	98.88	41–1	221	6	215	2.71	97.29
37–2	284	6	278	2.11	97.89	41–2	294	7	287	2.38	97.62
38–1	218	0	218	.00	100.00						
38–2	130	3	127	2.31	97.69	Total	4,728	60	4,668	1.27	98.73

MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE

44–1	324	178	146	54.94	45.06	46–3	27	23	4	85.19	14.81
44–2	148	86	62	58.11	41.89	46–4	68	43	25	63.24	36.76
44–3	223	117	106	52.47	47.53	47–1	59	37	22	62.71	37.29
44–4	419	219	200	52.27	47.73	47–2	57	38	19	66.67	33.33
45–1	367	188	179	51.23	48.77	47–3	162	99	63	61.11	38.89
46–1	27	18	9	66.67	33.33						
46–2	25	15	10	60.00	40.00	Total	1,906	1,061	845	55.67	44.33

The F_2 results from the reciprocal cross (resistant × susceptible) are presented in table 2. Of the 1,906 plants tested, 845 became diseased, and 1,061 remained healthy. This is a ratio of 55.67 percent resistant to 44.33 percent susceptible.

The results obtained in the second generation, though not so striking as the F_1 results, still show a preponderating influence of the grandmaternal parent. In the cross susceptible × resistant more than 90 percent of the plants were susceptible in every case. Likewise, in the cross resistant × susceptible more than 50 percent of the plants were resistant in every case. The result of 55.67 percent resistant and 44.33 percent susceptible when the cross is made this way (resistant × susceptible) becomes more significant if it is realized that in no family was there more than 48.77 percent susceptibility or less than 51.23 percent resistance. It should be noted that certain F_1 plants and F_2 progenies are identical reciprocals, i. e., from crosses between the same parent plants. For example, the F_1 plant 34–3 (table 1) is from a cross between parent 1–2 (Stringless Green Refugee) as female and parent 1–16 (Michigan Robust) as male. The F_1 individuals 45–2 and 45–3 (table 1) are from the reciprocal cross, parent 1–16 (Michigan Robust) × parent 1–2 (Stringless Green Refugee).

BEHAVIOR OF F_3 GENERATION OF MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE AND RECIPROCAL

The F_3 plants tested were descendants of self-pollinated F_2 individuals whose reaction to the virus had been determined in the second generation. The results of inoculating F_3 progenies have been grouped in tables 3 to 6, according to the original cross and the reaction of the F_2 parent.

TABLE 3.—*Reaction to common bean mosaic of F_3 generation of susceptible (Stringless Green Refugee) ♀ × resistant (Michigan Robust) ♂ and reciprocal, derived from plants resistant in F_2*

STRINGLESS GREEN REFUGEE × MICHIGAN ROBUST

Progeny no.	F_3 plants					Progeny no.	F_3 plants				
	Number			Percent			Number			Percent	
	Tested	Healthy	Diseased	Healthy	Diseased		Tested	Healthy	Diseased	Healthy	Diseased
35-1-1	32	21	11	65.63	34.37	39-1-1	205	31	174	15.12	84.88
35-1-2	147	81	66	55.10	44.90	39-2-1	11	6	5	54.55	45.45
36-1-1	33	30	3	90.91	9.09	39-2-2	12	5	7	41.67	58.33
36-1-2	50	50	0	100.00	.00	39-2-3	14	12	2	85.71	14.29
36-1-3	242	240	2	99.17	.83	40-3-1	12	10	2	83.33	16.67
36-1-4	28	27	1	96.43	3.57	41-1-1	18	18	0	100.00	.00
36-1-5	11	11	0	100.00	.00	41-1-2	86	74	12	86.05	13.95
36-2-1	356	348	8	97.75	2.25	41-1-3	176	170	6	96.59	3.41
36-2-2	132	111	21	84.09	15.91	41-1-4	90	74	16	82.22	17.78
36-2-3	244	186	58	76.23	23.77	41-1-5	161	156	5	96.89	3.11
36-2-4	26	17	9	65.38	34.62	41-1-6	77	76	1	98.70	1.30
36-2-5	10	4	6	40.00	60.00	41-1-7	16	16	0	100.00	.00
36-3-1	135	117	18	86.67	13.33	41-1-8	18	17	1	94.44	5.56
36-3-2	201	97	104	48.26	51.74	41-2-1	23	23	0	100.00	.00
36-3-3	289	283	6	97.92	2.08	41-2-2	29	27	2	93.10	6.90
36-3-4	56	50	6	89.29	10.71	41-2-3	33	28	5	84.85	15.15
37-1-1	70	69	1	98.57	1.43	41-2-4	22	17	5	77.27	22.73
37-1-2	97	89	8	91.75	8.25	41-2-5	214	206	8	96.26	3.74
37-2-1	110	103	7	93.64	6.36	41-2-6	23	19	4	82.61	17.39
37-2-2	5	4	1	80.00	20.00	41-2-7	182	179	3	98.35	1.65
37-2-3	8	6	2	75.00	25.00	41-2-8	22	19	3	86.36	13.64
37-2-4	12	12	0	100.00	.00	41-2-9	133	127	6	95.49	4.51
37-2-5	3	2	1	66.67	33.33	41-2-10	136	133	3	97.79	2.21
37-2-6	7	5	2	71.43	28.57	41-2-11	204	199	5	97.55	2.45
37-2-7	10	9	1	90.00	10.00						
37-2-8	4	2	2	50.00	50.00	Total	4,235	3,616	619	85.38	14.62

MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE

44-1-1	199	176	23	88.44	11.56	44-3-3	48	32	16	66.67	33.33
44-1-2	126	124	2	98.41	1.59	44-3-4	11	6	5	54.55	45.45
44-1-3	124	105	19	84.68	15.32	44-3-5	13	7	6	53.85	46.15
44-1-4	99	80	19	80.81	19.19	44-3-6	8	8	0	100.00	.00
44-1-5	82	82	0	100.00	.00	44-3-7	14	10	4	71.43	28.57
44-1-6	9	7	2	77.78	22.22	44-3-8	4	4	0	100.00	.00
44-1-7	6	3	3	50.00	50.00	44-3-9	7	4	3	57.14	42.86
44-1-8	3	2	1	66.67	33.33	44-3-10	4	2	2	50.00	50.00
44-1-9	4	4	0	100.00	.00	44-3-11	8	6	2	75.00	25.00
44-1-10	17	14	3	82.35	17.65	44-3-12	6	6	0	100.00	.00
44-1-11	6	4	2	66.67	33.33	44-4-1	30	28	2	93.33	6.67
44-1-12	16	9	7	56.25	43.75	44-4-2	33	28	5	84.85	15.15
44-1-13	8	5	3	62.50	37.50	44-4-3	132	107	25	81.06	18.94
44-2-1	18	14	4	77.78	22.22	44-4-4	7	3	4	42.86	57.14
44-2-2	13	7	6	53.85	46.15	44-4-5	8	3	5	37.50	62.50
44-2-3	8	6	2	75.00	25.00	44-4-6	5	5	0	100.00	.00
44-2-4	8	5	3	62.50	37.50	44-4-7	6	6	0	100.00	.00
44-2-5	7	5	2	71.43	28.57	44-4-8	5	2	3	40.00	60.00
44-2-6	14	12	2	85.71	14.29	45-1-1	22	14	8	63.64	36.36
44-2-7	8	8	0	100.00	.00	45-1-2	35	10	25	28.57	71.43
44-2-8	11	7	4	63.64	36.36	45-1-3	39	12	27	30.77	69.23
44-2-9	5	4	1	80.00	20.00	45-1-4	14	12	2	85.72	14.28
44-2-10	6	6	0	100.00	.00	45-1-5	17	14	3	82.35	17.65
44-2-11	7	6	1	85.72	14.29	45-1-6	10	5	5	50.00	50.00
44-2-12	8	4	4	50.00	50.00	45-1-7	8	3	5	37.50	62.50
44-2-13	11	8	3	72.73	27.27	46-4-1	11	5	6	45.45	54.55
44-2-14	9	5	4	55.56	44.44	47-2-1	7	6	1	85.71	14.29
44-2-15	8	6	2	75.00	25.00	47-2-2	7	4	3	57.14	42.86
44-2-16	16	9	7	56.25	43.75	47-2-3	4	2	2	50.00	50.00
44-2-17	7	4	3	57.14	42.86	47-2-4	12	12	0	100.00	.00
44-2-18	5	5	0	100.00	.00	47-2-5	6	6	0	100.00	.00
44-2-19	6	6	0	100.00	.00						
44-3-1	45	23	22	51.11	48.89	Total	1,488	1,159	329	77.89	22.11
44-3-2	28	22	6	78.57	21.43						

Table 3 shows the results of inoculating F_3 progenies derived from resistant F_2 plants from the cross susceptible (Stringless Green Refugee) $\times$ resistant (Michigan Robust). The following distribution of 50 F_3 progenies was obtained: In 6 (12 percent) of the progenies, all plants were resistant; in 13 (26 percent), 95 to 99 percent of the plants were resistant; in 20 (40 percent), 75 to 94 percent were resistant; in 7 (14 percent), 50 to 74 percent were resistant; and in 4 (8 percent), less than 50 percent were resistant. In this case 38 percent (19 out of 50) of the progenies contained more than 95 percent resistant plants, and 40 percent (20 out of 50) contained from 75 to 94 percent resistant plants.

There were 65 F_3 progenies from resistant F_2 plants of the reciprocal cross (Michigan Robust $\times$ Stringless Green Refugee) available for testing. The results of inoculating these progenies with the mosaic virus are presented in table 3. The distribution in this case is markedly different from the distribution in the material described above. Thirteen (20 percent) of the progenies were 100 percent resistant, 1 (1.54 percent) was 95 to 99 percent resistant, 19 (29 percent) were 75 to 94 percent resistant, 25 (38 percent) were 50 to 74 percent resistant, and 7 (11 percent) contained less than 50 percent resistant plants.

Of the 4,235 plants tested (F_3 susceptible $\times$ resistant from plants resistant in F_2) 3,616 were healthy and 619 were diseased, a ratio of 85.38 percent resistant to 14.62 percent susceptible. Of the 1,488 plants tested of the reciprocal cross (F_3 resistant $\times$ susceptible from plants resistant in F_2) 1,159 were healthy and 329 diseased, a ratio of 77.89 percent resistant to 22.11 percent susceptible.

Table 4 (a summary of table 3) gives the percentages of F_3 progenies, obtained from resistant F_2 plants, which fall into the various divisions arbitrarily based upon the percentage of resistant plants in a progeny.

TABLE 4.—*Summary of table 3*

F_3 plants resistant (percent)	F_3 progenies resistant (from F_2 resistant plants of indicated cross)	
	Susceptible $\times$ resistant	Resistant $\times$ susceptible
	Percent	*Percent*
95 to 100	38	22
75 to 100	78	51
50 to 100	92	89
0 to 50	8	11

From the results obtained in the F_1 and F_2 generations of crosses between Michigan Robust and Stringless Green Refugee varieties it might be expected that the resistant plants from the Michigan Robust $\times$ Stringless Green Refugee cross would produce and maintain a high degree of resistance, but this is apparently not the case. The progeny test indicates that the F_2 plants of the cross Stringless Green Refugee $\times$ Michigan Robust that were designated as resistant differed in composition from at least some of the resistant plants from the reciprocal cross.

In table 5 are presented the results of inoculating F_3 progenies of plants of the Stringless Green Refugee × Michigan Robust cross that were susceptible in the F_2 generation.

TABLE 5.—*Reaction to common bean mosaic of F_3 generation of susceptible (Stringless Green Refugee) ♀ × resistant (Michigan Robust) ♂, derived from plants susceptible in F_2*

STRINGLESS GREEN REFUGEE × MICHIGAN ROBUST

Progeny no.	F_3 plants					Progeny no.	F_3 plants				
	Number			Percent			Number			Percent	
	Tested	Healthy	Diseased	Healthy	Diseased		Tested	Healthy	Diseased	Healthy	Diseased
40-1-1	288	17	271	5.90	94.10	40-4-1	169	11	158	6.51	93.49
40-1-2	148	9	139	6.08	93.92	40-4-2	115	3	112	2.61	97.39
40-1-3	98	2	96	2.04	97.96	40-4-3	120	3	117	2.50	97.50
40-1-4	66	1	65	1.51	98.49	40-4-4	49	5	44	10.20	89.80
40-1-5	70	1	69	1.43	98.57	40-4-5	59	4	55	6.78	93.22
40-1-6	87	0	87	.00	100.00	40-4-6	131	10	121	7.63	92.37
40-1-7	40	2	38	5.00	95.00	40-4-7	32	3	29	9.37	90.63
40-1-8	44	4	40	9.09	90.91	41-1-9	21	1	20	4.76	95.24
40-1-9	61	2	59	3.28	96.72						
40-1-10	16	0	16	.00	100.00	Total	1,627	79	1,548	4.86	95.14
40-1-11	13	1	12	7.69	92.31						

MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE

44-1-6	36	1	35	2.78	97.22	44-4-4	43	17	26	39.53	60.47
44-1-7	18	2	16	11.11	88.89	44-4-5	33	3	30	9.09	90.91
44-1-8	131	24	107	18.32	81.68	45-1-4	34	4	30	11.76	88.24
44-1-9	25	10	15	40.00	60.00	45-1-5	24	8	16	33.33	66.67
44-1-10	16	6	10	37.50	62.50						
44-3-4	91	52	39	57.14	42.86	Total	451	127	324	28.16	71.84

Of the 19 progenies tested, 2 (10 percent) were 100 percent susceptible, 8 (42 percent) were 95 to 99 percent susceptible, and 9 (47 percent) contained 90 to 94 percent of susceptible plants. These results are in close agreement with what would be expected from susceptible F_2 plants. Out of 1,627 plants tested, 79 were healthy and 1,548 were diseased, i. e., 4.86 percent were resistant and 95.14 percent were susceptible.

Ten progenies derived from F_2 susceptible plants of the cross Michigan Robust × Stringless Green Refugee (table 5) gave the following distribution: 1 progeny contained 97 percent of susceptible plants, 1 contained 91 percent, 3 contained 80 to 90 percent, and 5 contained 43 to 67 percent of susceptible plants. Out of 451 plants tested 127 were healthy and 324 were diseased, i. e., 28.16 percent were resistant and 71.84 percent were susceptible.

It is evident from these results that F_3 progenies derived from susceptible F_2 individuals of the cross resistant × susceptible contained more resistant plants than F_3 progenies derived from susceptible F_2 plants of the reciprocal cross.

In table 6 are presented the results obtained when progenies were kept disease-free in the F_1 and F_2 generations and inoculated with the common bean mosaic virus in the F_3 generation.

TABLE 6.—*Reaction to common bean mosaic of F_3 generation of susceptible (Stringless Green Refugee) ♀ × resistant (Michigan Robust) ♂ and reciprocal, derived from plants uninoculated in F_1 and F_2*

STRINGLESS GREEN REFUGEE × MICHIGAN ROBUST

Progeny no.	F_3 plants					Progeny no.	F_3 plants				
	Number			Percent			Number			Percent	
	Tested	Healthy	Diseased	Healthy	Diseased		Tested	Healthy	Diseased	Healthy	Diseased
35-1-1	6	0	6	0.00	100.00	37-2-2	7	1	6	14.28	85.72
35-1-2	14	2	12	14.29	85.71	37-2-3	10	8	2	80.00	20.00
35-1-3	12	0	12	.00	100.00	39-1-1	42	0	42	.00	100.00
35-1-4	8	2	6	25.00	75.00	39-1-2	8	0	8	.00	100.00
35-1-5	12	0	12	.00	100.00	39-1-3	4	0	4	.00	100.00
35-1-6	4	0	4	.00	100.00	39-1-4	2	0	2	.00	100.00
36-1-1	10	2	8	20.00	80.00	39-1-5	25	1	24	4.00	96.00
36-1-2	8	0	8	.00	100.00	39-1-6	21	1	20	4.76	95.24
36-1-3	2	0	2	.00	100.00	39-1-7	16	0	16	.00	100.00
36-1-4	8	2	6	25.00	75.00	39-2-1	9	0	9	.00	100.00
36-2-1	12	0	12	.00	100.00	39-2-2	10	1	9	10.00	93.00
36-2-2	22	6	16	27.27	72.73	39-2-3	11	0	11	.00	100.00
36-2-3	12	2	10	16.67	83.33	39-2-4	18	2	16	11.11	88.89
36-2-4	10	8	2	80.00	20.00	41-2-1	19	1	18	5.26	94.74
36-2-5	12	2	10	16.67	83.33	41-2-2	35	1	34	2.86	97.14
36-3-1	16	2	14	12.50	87.50	41-2-3	9	0	9	.00	100.00
36-3-2	18	0	18	.00	100.00						
36-3-3	28	2	26	7.14	92.86	Total	492	46	446	9.35	90.65
36-3-4	26	0	26	.00	100.00	F_2 result				1.27	98.73
37-2-1	6	0	6	.00	100.00	F_1 result				.00	100.00

MICHIGAN ROBUST × STRINGLESS GREEN REFUGEE

44-2-1	8	4	4	50.00	50.00	45-4-1	9	0	9	0.00	100.00
44-2-2	10	6	4	60.00	40.00	46-4-2	11	2	9	18.18	81.82
44-2-3	4	2	2	50.00	50.00	45-4-3	9	5	4	55.56	44.44
44-2-4	3	1	2	33.33	66.67	45-4-4	6	0	6	.00	100.00
44-3-1	7	3	4	42.86	57.14	47-2-1	8	2	6	25.00	75.00
44-3-2	8	2	6	25.00	75.00	47-2-2	14	3	11	21.43	78.57
44-3-3	10	7	3	70.00	30.00	47-2-3	22	10	12	45.45	54.55
44-4-1	13	7	6	53.85	46.15	47-2-4	9	3	6	33.33	66.67
44-4-2	6	2	4	33.33	66.67	47-2-5	8	2	6	25.00	75.00
45-1-1	8	1	7	12.50	87.50						
45-1-2	9	5	4	55.56	44.44	Total	213	74	139	34.74	65.26
45-1-3	6	4	2	66.67	33.33	F_2 result				55.67	44.33
45-1-4	14	2	12	14.29	85.71	F_1 result				82.35	17.65
45-1-5	11	1	10	9.09	90.91						

Table 6 gives the data obtained from 36 tested F_3 progenies of susceptible × resistant crosses. Eighteen (50 percent) of the progenies were 100 percent susceptible, 5 (14 percent) were 91 to 99 percent susceptible, 7 (19 percent) were 81 to 90 percent susceptible, 4 (11 percent) were 71 to 80 percent susceptible, and 2 (6 percent) were 20 percent susceptible. The last two progenies mentioned were the only ones that gave any considerable number of resistant plants. Of 492 plants tested, 446 (91 percent) were susceptible and 46 (9 percent) were resistant.

Twenty-three F_3 progenies of the reciprocal cross (table 6) gave the following distribution: Two (9 percent) of the progenies were 100 percent susceptible, 1 (4 percent) was 91 percent, 3 (13 percent) were from 80 to 89 percent, 4 (17 percent) were from 70 to 79 percent, 3 were from 60 to 69 percent, 4 were from 50 to 59 percent, 4 were from 40 to 49 percent, and 2 were from 30 to 39 percent susceptible. Of the 213 plants tested, 74 (35 percent) were resistant and 139 (65

percent) were susceptible. These results tend to verify the F_1 and F_2 results indicating that the crosses involving susceptible maternal parents produce a preponderance of susceptible offspring, and vice versa. When no inoculations were made until the F_3 generation the effect of the great-grandmaternal parent was still very evident though not so pronounced as in the F_1 and F_2 generations. It is of interest to note how the effect of the original maternal parent diminishes in each succeeding generation when the progenies are kept disease-free until they reach the generation which is to be studied. This is clearly shown in the results given in table 7.

TABLE 7.—*Summary of tables 1, 2, and 6*

Generation	Susceptible × resistant		Resistant × susceptible	
	Resistant	Susceptible	Resistant	Susceptible
	Percent	*Percent*	*Percent*	*Percent*
F_1	0.00	100.00	82.35	17.65
F_2	1.27	98.73	55.67	44.33
F_3	9.35	90.65	34.74	65.26

It is readily apparent, from the results shown in table 7, that the reciprocal crosses behave very differently. When the susceptible variety was used as the female parent the progenies remained over 90 percent susceptible even in the F_3 generation; however, the reciprocal cross was not nearly so stable in regard to mosaic resistance. The progenies changed from 82 percent of resistant plants in the F_1 generation to 35 percent of resistant plants in the F_3 generation. As may be seen from these results, there is a convergence of the reactions from generation to generation. The cross susceptible × resistant converges slowly, whereas the reciprocal (resistant × susceptible) converges more rapidly. It might be possible that the reciprocal crosses would reach the same equilibrium at some point in succeeding generations.

BEHAVIOR OF CORBETT REFUGEE × STRINGLESS GREEN REFUGEE AND RECIPROCAL

Corbett Refugee × Stringless Green Refugee gave all resistant F_1 plants, five individuals being tested. When the susceptible variety (Stringless Green Refugee) was used as the female parent the F_1 plants were again resistant. Extracts of the 10 plants tested, inoculated to disease-free susceptible plants, gave negative results, indicating that these plants were probably not carrying the virus in a masked condition.

The results obtained in the F_2 generation of Corbett Regufee × Stringless Green Refugee show that of the 124 plants tested 113 were healthy and 11 were diseased, a ratio of 91.13 percent resistant to 8.87 percent susceptible. In the reciprocal cross (Stringless Green Refugee × Corbett Refugee) (table 8) there were 269 healthy and 73 diseased in the 342 plants tested, or 78.65 percent resistant to 21.34 percent susceptible.

TABLE 8.—*Reaction to the common bean mosaic of F_2 generation of resistant (Corbett Refugee) ♀ × susceptible (Stringless Green Refugee) ♂ and reciprocal*

CORBETT REFUGEE × STRINGLESS GREEN REFUGEE

Progeny no.	F_2 plants					Progeny no.	F_2 plants				
	Number			Percent			Number			Percent	
	Tested	Healthy	Diseased	Healthy	Diseased		Tested	Healthy	Diseased	Healthy	Diseased
209–1	33	26	4	86.67	13.33	309–3	2	2	0	100.00	0.00
209–2	52	45	7	86.54	13.46	Total	124	113	11	91.13	8.87
309–1	19	19	0	100.00	.00						
309–2	21	21	0	100.00	.00						

STRINGLESS GREEN REFUGEE × CORBETT REFUGEE

210–1	52	32	20	61.54	38.46	313–1	31	26	5	83.87	16.13
210–2	44	28	16	63.64	36.36	313–2	52	45	7	86.54	13.46
210–3	51	38	13	74.51	25.49	314–1	14	14	0	100.00	.00
311–1	20	18	2	90.00	10.00	314–2	23	23	0	100.00	.00
311–2	10	10	0	100.00	.00						
312–1	45	35	10	77.78	22.22	Total	342	269	73	78.65	21.35

The reciprocals in this cross are unlike, but the dissimilarity is not as great as it is when Michigan Robust is used as the resistant parent. The results are still apparently non-Mendelian. They do not conform to any Mendelian ratio, although the results obtained when Stringless Green Refugee is used as the female parent do approach a 3 : 1 ratio (observed, 79 : 21 percent; calculated, 75 : 25 percent).

BEHAVIOR OF CORBETT REFUGEE × MICHIGAN ROBUST AND RECIPROCAL

Crosses between the two resistant varieties have yielded all resistant F_1 plants. Eleven plants from the cross Michigan Robust × Corbett Refugee and five plants from the cross Corbett Refugee × Michigan Robust have been tested. The F_1 plants were self-pollinated to obtain material for an F_2 generation.

Table 9 shows the results obtained in the F_2 generation of Michigan Robust × Corbett Refugee. There were 729 plants tested, 663 being healthy and 66 becoming diseased (91 and 9 percent, respectively). In the reciprocal cross (table 9) 374 plants were tested, 349 being resistant and 25 being susceptible (93 and 7 percent, respectively).

The reciprocal crosses in this case reacted similarly to inoculations with the virus. However, the occurrence of susceptible plants in these crosses is unexpected, and if an explanation is possible for such facts it should help to make the findings reported in this paper more understandable.

TABLE 9.—*Reaction to common bean mosaic of F_2 generation of resistant (Michigan Robust) ♀ × resistant (Corbett Refugee) ♂ and reciprocal*

MICHIGAN ROBUST × CORBETT REFUGEE

Progeny no.	F_2 plants					Progeny no.	F_2 plants				
	Number			Percent			Number			Percent	
	Tested	Healthy	Diseased	Healthy	Diseased		Tested	Healthy	Diseased	Healthy	Diseased
301–1	166	147	19	88.55	11.45	304–2	40	40	0	100.00	0.00
301–2	86	80	6	93.02	6.98	305–1	37	34	3	91.89	8.11
302–1	154	143	11	92.86	7.14	305–2	42	36	6	85.71	14.29
302–2	65	61	4	93.85	6.15	305–3	26	23	3	88.46	11.54
303–1	49	48	1	97.96	2.04						
303–2	31	23	8	74.19	25.81	Total	729	663	66	90.95	9.05
304–1	33	28	5	84.85	15.15						

CORBETT REFUGEE × MICHIGAN ROBUST

306–1	100	95	5	95.00	5.00	308–2	13	13	0	100.00	0.00
306–2	134	126	8	94.03	5.97						
307–1	105	94	11	89.52	10.48	Total	374	349	25	93.32	6.68
308–1	22	21	1	95.45	4.55						

NATURE OF THE RESISTANCE

With the object of studying the nature of immunity in the resistant varieties, two plants of the Stringless Green Refugee variety just coming into blossom in the greenhouse were inoculated with juice extracted from Michigan Robust plants and two individuals were treated with juice extracted from Corbett Refugee plants. The seed of these plants was harvested and planted in the greenhouse bench. The seedlings were treated at 10-day intervals. Plants 1 to 8 were inoculated in the two-leaf stage with juice extracted from Michigan Robust plants, and plants 9 to 12 were inoculated with juice extracted from Corbett Refugee. After 10 days the second treatment was given. Plants 1 to 4 were then inoculated with the mosaic virus, and plants 5 to 8 were reinoculated with juice from the resistant Michigan Robust. Likewise, plants 9 and 10 were inoculated with virus and plants 11 and 12 were reinoculated with Corbett Refugee juice. In the third treatment, plants 5 and 6 were given the virus and plants 7 and 8 were given additional Michigan Robust juice; plant 11 was inoculated with the virus extract and plant 12 was reinoculated with juice from Corbett Refugee. The fourth treatment, given 30 days after the first, consisted of inoculating plants 7, 8, and 12 with extract from mosaic-diseased plants. All the plants developed mosaic symptoms within a short period following inoculation with the virus. These results suggest that the immunity of the Michigan Robust and Corbett Refugee varieties is not transferred to susceptible varieties by this treatment.

The effect of grafting was also studied. Reciprocal grafts were made between Michigan Robust and Stringless Green Refugee, and between Corbett Refugee and Stringless Green Refugee, whip grafts, cleft grafts, and inarching being employed. The results of this experiment are shown in table 10. In no case was the reaction of the scion altered by contact with the stock. Likewise, foliage produced on the stock

was not changed by the presence of a scion from a different variety. Both symbionts reacted to the virus as if they were growing as separate plants.

TABLE 10.—*Results of grafting*

Stock	Scion	Type of graft	Reaction of scion to virus
Michigan Robust	Stringless Green Refugee	Inarch	Susceptible.
Do	do	Whip	Do.
Do	do	do	Do.
Do	do	Cleft	Do.
Stringless Green Refugee	Michigan Robust	Whip	Resistant.
Do	do	Cleft	Do.
Corbett Refugee	Stringless Green Refugee	Inarch	Susceptible.
Do	do	Cleft	Do.
Do	do	do	Do.
Do	do	do	Do.
Stringless Green Refugee	Corbett Refugee	do	Resistant.
Do	do	do	Do.
Do	do	Whip	Do.

DISCUSSION

It is evident that the results reported here cannot be accounted for by a simple Mendelian explanation. In Mendelian types of inheritance reciprocal crosses usually react alike. The exceptions are cases of sex linkage, etc., which have no bearing on the present problem. In examining the results obtained upon inoculating progenies from Stringless Green Refugee × Michigan Robust crosses and reciprocals, it is found that the maternal parent determines to a large extent the reaction of the hybrid individuals. In the F_1 generation this influence is very marked; in the F_2 generation the effect is still very noticeable; in the F_3 generation the effect is not as marked as in the preceding generations but is still conspicuous. However, the effect of the pollen parent must not be overlooked. The influence of the paternal parent was evident in 3 out of 17 plants tested in the F_1 generation of Michigan Robust × Stringless Green Refugee; it was not shown in the reciprocal cross in F_1. The effect of the pollen parent was more evident in all cases in the F_2 and F_3 generations.

F_1 plants from the cross Stringless Green Refugee × Corbett Refugee manifest the influence of the pollen parent in a significant way. F_2 individuals from reciprocal crosses between Stringless Green Refugee and Michigan Robust show a preponderant influence of the grandmaternal parent. Likewise, when Corbett Refugee was used as the resistant parent more resistant F_2 individuals were obtained if the Corbett Refugee plants had been used as grandmaternal parents.

To explain the results obtained in reciprocal crosses between Michigan Robust and Stringless Green Refugee it is necessary to assume a cytoplasmic or extranuclear influence. It is also necessary to assume that a portion of the cytoplasm or particular inclusions accompanies the male gametes at the time of fertilization. The suggestion that biparental cytoplasmic inheritance occurs in the bean is substantiated by the results obtained in the writer's studies on a variegated leaf character (*15*).

In order to obtain independent evidence of the role played by the cytoplasm in heredity in beans, two distinct chlorophyll-deficient types were investigated. One of these, termed "yellow spot", was found

to behave in a simple Mendelian manner (*14*); the other, called "variegated", provided direct evidence of inheritance through the cytoplasm (*15*). Below is a brief summary and discussion of the results with the variegated character.

All F_1 plants from the variegated × normal green cross were variegated. One out of eight F_1 plants from the reciprocal cross (normal green × variegated) showed one small variegated area; the remaining seven plants were green.

In the second generation of the variegated × normal green cross, 84 percent variegated and 16 percent normal green plants were obtained. In the reciprocal (normal green × variegated) 93 percent of normal green and 7 percent of variegated plants were found. These results suggest that the variegated condition is governed by the character of the cytoplasm and that the causal agency is introduced with the pollen tube at the time of fertilization. The production of variegated offspring by plants normal green in F_1 can be explained by assuming a segregation or sorting out of the material which causes the condition during growth and cell division in the F_1 plants and its inclusion in such proportions in some of the F_2 ovules that it comes to expression in the developing individual. The occurrence of a slight variegation in a leaf of one plant in the F_1 generation of normal green × variegated might be explained by assuming that a sufficient amount of the cytoplasm (or its inclusions) was introduced into the egg from the pollen parent to eventually influence a small area of the F_1 individual.

The explanation, as outlined above, is similar to that reported by Baur (*1*, *2*) and later by Noack (*11*, *12*, *13*), for the Albomarginata variety of *Pelargonium zonale* Willd. This form, characterized by leaves with white margins, produces sometimes pure-white and sometimes pure-green branches. Reciprocal crosses between flowers on these two kinds of branches may result in variegated offspring. Baur explains the appearance of green and white tissue by assuming the presence of two kinds of plastids or plastid primordia, which are sorted out during cell division, one type of primordium giving rise to green plastids, the other to white or colorless plastids. Baur states that these plastid primordia are contributed by both parents.

Winge (*21*) suggests that in those cases where variegated plants occur the cytoplasm in which the plastids lie is responsible for the defect. According to Winge the two classes of non-Mendelian inheritance, maternal and biparental, are due to the fact that in some instances paternal cytoplasm is introduced into the egg with the male gametes whereas in others it is not.

Noack (*10*, *11*, *12*) also studied *Pelargonium zonale*. He obtained essentially the same results as did Baur, except in crosses with green plants. Baur reported similar results in reciprocal crosses with pure-green plants, but in the case of the material used by Noack a larger proportion of variegated plants was obtained when *zonale* plants were used as female parents. In crosses with green, different *zonale* plants gave rise to different proportions of variegated plants and also different intensities of variegation.

According to Demerec (*4*) neither Baur's nor Noack's conclusions have sufficient support. He states (*4*, *p. 151*):

> Results identical with those obtained in the experiments would be expected if it is assumed that the *zonale* character is determined by a dominant gene which

becomes mutable (mutating to the gene for green) in crosses with green, or when the plant is heterozygous for green. Such an assumption would be supported by similar behavior observed in several other cases.

He then discusses the work of Emerson (*5*) on variegated pericarp in maize in which Emerson states that the mutability of the gene for variegated pericarp is increased when the gene is in a heterozygous condition.

Demerec further states (*4, p. 152*):

> The fact, observed by Noack, that reciprocal crosses between *zonale* and green gave different results, could be explained by the assumption that the mutability of the gene for white is increased when brought by the pollen nucleus into other than maternal cytoplasm.

Essentially, Demerec's hypothesis involves the assumption that the cytoplasm exerts an indirect effect by first influencing the gene, while the earlier explanations (*1, 2, 21*) assume a direct influence of the cytoplasm. Demerec grants the non-Mendelian inheritance of a chlorophyll character in maize which is only transmitted through the maternal parent. It is possible that in certain forms the cytoplasm or other extranuclear material accompanies the male gamete into the egg and in such cases biparental non-Mendelian inheritance could be explained without the necessity of assuming a high mutability of the gene when in foreign cytoplasm.

The behavior of the variegated character can be explained entirely on a non-Mendelian or cytoplasmic basis, but the cytoplasmic explanation does not cover all the facts obtained in the studies on the inheritance of resistance to the mosaic virus. Apparently the immediate reaction of the plant is determined by the character of the cytoplasm, but the nature of the cytoplasm ultimately depends upon nuclear factors. Therefore, the phenomenon may be primarily nuclear though overshadowed by the effect of the cytoplasm. We must assume that certain genotypes change the reaction of the cytoplasm more rapidly than others. Such an explanation will account for the difference in reciprocals and for the convergence which occurs in the F_2 and F_3 generations.

Considerable irregularity occurs in all generations of the cross Stringless Green Refugee × Michigan Robust and reciprocal. The fact that the reciprocal crosses react differently in each generation suggests a strong cytoplasmic influence on the inheritance of resistance. For example, the F_3 generation of Michigan Robust × Stringless Green Refugee showed more susceptible plants than resistant ones in the material which had been kept disease-free until that time, and, in comparison with the reciprocal cross, a higher percentage of resistant plants from plants susceptible in F_2 and a higher percentage of susceptible plants from plants resistant in F_2. Throughout the studies of this cross (Michigan Robust × Stringless Green Refugee) neither resistant nor susceptible plants have remained stable. Some resistant and some susceptible plants have been isolated which produce only resistant and susceptible plants, respectively, but the majority of the plants, as may be seen from the F_3 tables, produce some plants of the opposite reaction in the following generation. This inconsistency can also be explained by assuming that the distribution or behavior of the cytoplasmic units responsible for the resistance, or governing the action of genes for resistance, is not as regular or as systematic in cell division as is the distribution of nuclear factors.

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It is apparent that the two resistant varieties used in this investigation differ in the type of resistance carried or that the resistance is governed by different heritable entities in the two. At least they differ in ability to transmit this immunity in crosses with susceptible plants.

When Michigan Robust was used as the male parent and Stringless Green Refugee as the female parent all the F_1 plants were susceptible and in the F_3 generation 90 percent of the plants were still susceptible. However, plants that were resistant in the F_2 generation maintained a high degree of resistance in the F_3 generation. When Corbett Refugee was used as the male parent in crosses with Stringless Green Refugee all the F_1 plants were resistant and 79 percent were resistant in the F_2 generation.

The appearance of susceptible plants in the F_2 generation of crosses between the two resistant varieties is further proof that these two varieties differ in their heritable bases for resistance. Corbett Refugee was developed from a single resistant plant in a field of severely infected Stringless Green Refugee beans. If it is assumed that a dominant gene mutation occurred in this case, the following explanation is possible. Let *R* represent the genetic factor for resistance and *r* the genetic factor for susceptibility. The cytoplasm of Corbett Refugee in the absence of the genes for resistance is susceptible (*i*) to the virus, and the cytoplasm of Michigan Robust is immune (*I*) to the virus. The assumed genetic and cytoplasmic constitution of Corbett Refugee is *RR*(*i*), and of Michigan Robust, *rr*(*I*). When these two varieties are crossed the F_1 is resistant, owing almost if not entirely to the genetic factor *R* when Corbett Refugee is used as the female parent, and to the presence of the resistant factor and also resistant cytoplasm (*I*) when Michigan Robust is used as the female parent. In the F_2 generation, with a monohybrid segregation, we obtain 1 *RR* : 2 *Rr* : 1 *rr*. However, contrary to expectation, the homozygous recessive individuals are not all susceptible, because some of these individuals have received resistant cytoplasmic factors from the Michigan Robust parent. Therefore we have a deficiency in the homozygous recessive class no matter which way the cross is made.

The above assumption that Corbett Refugee carries genetic factors for resistance does not completely explain the results obtained in the reciprocal crosses between Corbett Refugee and Stringless Green Refugee. If the genetic constitution of Corbett Refugee is *RR* and that of Stringless Green Refugee is *rr*, then the F_1 should be resistant and a ratio of 3 resistant to 1 susceptible should be obtained in F_2. The F_1 results obtained are as expected. In the F_2 the results obtained are not in agreement with the expected results, Corbett Refugee × Stringless Green Refugee (observed, 113 : 11; calculated on 3 : 1 basis, 93 : 31), deviating by 20 from the expected, and Stringless Green Refugee × Corbett Refugee (observed, 269 : 73; calculated on 3 : 1 basis, 256.5 : 85.5), deviating by 12.5 from the expected, in favor of the resistant class. The numbers involved in this cross are small, and it is possible that if larger numbers were available the results would more closely approximate a 3 : 1 ratio. It is not improbable, however, that there is some other underlying reason for the occurrence of a larger number of resistant plants than was to be expected under the explanation just given.

The complete explanation and understanding of the foregoing results may be linked with an explanation and understanding of the existence of so-called "tolerant" bean varieties, or varieties which are fairly easily infected with the virus but which do not ordinarily develop symptoms that can be readily identified. It is difficult to study progenies from crosses between tolerant varieties and either susceptible or resistant varieties, because of the difficulty in distinguishing the tolerant plants in a hybrid population.

As the complex plant viroses become better understood a technique may be developed whereby crosses between tolerant and resistant (or susceptible) varieties can be studied. Such crosses should give further evidence on the method of inheritance of resistance to the common mosaic virus in the bean.[7]

SUMMARY

Reciprocal crosses between the mosaic-resistant Michigan Robust and susceptible Stringless Green Rufugee varieties of beans reacted quite differently to the mosaic virus employed in these studies. It was found that the maternal parent governs to a large extent the reaction of the hybrid offspring.

In the F_1 generation, all plants were susceptible from the cross Stringless Green Rufugee ♀ × Michigan Robust ♂, and 82 percent were resistant from the cross Michigan Robust × Stringless Green Refugee.

The influence of the grandmaternal parent was still very evident in the F_2 generation. From the cross Stringless Green Refugee × Michigan Robust, 99 percent of the F_2 individuals were susceptible to mosaic, whereas 56 percent of the individuals from the reciprocal cross were resistant.

In the F_3 generation the reciprocal crosses were still very different. From the cross susceptible × resistant, 91 percent of the individuals were susceptible and 9 percent were resistant. In the reciprocal cross (resistant × susceptible), 35 percent of the F_3 offspring were resistant and 65 percent were susceptible. Throughout the three generations studied there is evidenced a very marked convergence of results.

By studying F_3 individuals derived from plants of known reaction in F_2, it was found that some plants classified as resistant in F_2 gave rise to both susceptible and resistant plants in F_3 and also that plants susceptible in F_2 gave rise to some resistant plants in F_3.

These results cannot be explained on a simple Mendelian basis, because the reciprocal hybrids react differently. To explain the fact that the maternal parent determines to a large degree the reaction of the hybrid individuals it is assumed that the cytoplasm or some extranuclear inclusion governs the immediate reaction of the plant to the virus. The convergence of the results from reciprocal crosses in the F_2 and F_3, however, points to the conclusion that the ultimate control is nuclear but that there is a delayed expression of the action of the genes. The further assumption is necessary that certain genotypes change the reaction of the cytoplasm more rapidly than others.

[7] The writer has found no parallel in the literature of plant genetics to the behavior described here for the inheritance of resistance to common bean mosaic. Since this paper was submitted for publication, however, his attention has been drawn to an apparently similar case in mice studied by Little (8). Little found a marked difference in reciprocal crosses between a high tumor line and a low tumor line, and there is an indication, at least, of convergence of the reciprocals in F_2.

Corbett Refugee, a second resistant variety, was used in crosses with susceptible Stringless Green Refugee and with resistant Michigan Robust. The F_1 generation of Corbett Refugee × Stringless Green Refugee and the F_1 generation of the reciprocal were indentical, all individuals being resistant. In the F_2 generation there was a difference in the reciprocal crosses. From the cross Corbett Refugee × Stringless Green Refugee 91 percent of the F_2 individuals were resistant and 9 percent were susceptible. In the reciprocal cross 79 percent were resistant and 21 percent were susceptible. These results are apparently non-Mendelian also; they do not conform to any Mendelian ratio, although the results obtained when Stringless Green Refugee is used as the female parent do approach a 3 : 1 ratio.

Crosses between the two resistant varieties yielded all resistant F_1 plants. In the F_2 generation, however, there were 7 percent susceptibles from Corbett Refugee × Michigan Robust and 9 percent susceptibles from Michigan Robust × Corbett Refugee. From these results it is quite evident that the two resistant varieties differ in their bases for resistance, or at least that they differ in ability to transmit this resistance in crosses with susceptible plants.

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RELATION OF STOMATAL FUNCTION OF WHEAT TO INVASION AND INFECTION BY LEAF RUST (PUCCINIA TRITICINA)[1]

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INTRODUCTION

Experience in culturing leaf rust of wheat (*Puccinia triticina* Eriks.) has shown that abundant infection of the host follows inoculations made in totally dark humidity chambers. Loftfield (*8*)[2] and Hart (*6*) found that stomata of wheat close and remain closed in darkness, while Hart (*6*) reported that stem rust (*P. graminis tritici* Eriks. and Henn.) cannot enter closed stomata. If, therefore, the stomata of the wheats used in studying leaf rust reacted as did those reported by Loftfield and Hart, it would be apparent either that leaf rust, in contrast to stem rust, can enter a completely closed stoma, or that closure commonly is insufficiently complete to exclude the entry hyphae of this rust. Studies were undertaken to afford information on this and related questions. A preliminary report on these investigations has been published (*4*).

MATERIAL AND METHODS

In the first section of this work the number of entries of the fungus into the host was judged indirectly by the number of pustules appearing on leaves within 10 days after inoculation. Parallel inoculation trials were conducted in a lighted and in a dark humidity chamber, which were held in a common water bath at a relatively constant temperature of 21° C. ±1°. The lighted chamber received sunlight filtered through a tray of running water, with supplementary illumination from a 500-watt incandescent lamp suspended 12 inches above the plants on cloudy days.

Inoculation methods were adapted to provide as uniform distribution of inoculum as possible simultaneously over the entire series of plants used in a single experiment. In the experiments with seedlings the first leaf of each plant was used exclusively, while younger leaves were excised before inoculation. For each variety, 10 leaves were inoculated in light and 10 in darkness. The plants to be used were grouped together on the floor, and rust spores were showered upon them by shaking heavily infected plants above them at a height of 2 to 3 feet. They were atomized with water in the evening and placed in the humidity chambers for 24 hours, where they were kept continuously moist by repeated atomizing. They were then removed to the greenhouse bench to dry off either in darkness or in very weak light. Records of infection were made after about 10 days either by counting the pustules and measuring the length of each leaf or by estimating the

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[2] Reference is made by number (italic) to Literature Cited, p 932.

percentage infection by the scale adopted by the United States Department of Agriculture.

Where the pustules per leaf were counted, the standard errors for an inoculation series comprising several varieties were computed by obtaining the deviations from the mean of each variety. A common standard error for each inoculation trial was calculated for each group in the dark and in the illuminated humidity chambers.

In a part of this study, entry was studied directly by microscopic examination of the epidermis of the first seedling leaf, a portion of which was stripped from both the dorsal and the ventral leaf surfaces after the inoculated leaf had been held in the humidity chambers the length of time required for spore germination and for the formation of appressoria or substomatal vesicles. The dorsal surface was more frequently used because of the greater facility in removing the epidermis. A little practice made it possible to secure strips of epidermis the width of the entire leaf and from 1 to 2 inches long. These strips were immediately immersed in absolute alcohol containing the stain fast green. Lloyd (*7*) showed that by this technique the stomatal apertures could be preserved exactly as they were before removal of the epidermis. This was confirmed in the present study by careful observation and counts on marked sections of leaf before and after removal and fixation of the epidermis. By application of Lloyd's technique the relation of rust entry to stomatal aperture was studied directly. Fast green, an alcohol-soluble dye, was particularly suited to the study, since it stains the fungus more deeply than the host tissue. In such preparations the stomatal aperture, appressoria, substomatal vesicles, and infecting hyphae could be clearly seen. Gibson (*5*) stripped off the epidermis of leaves of various plants to study the entry of rusts into species other than their own hosts, but gave no consideration to stomatal function.

The cultures of leaf rust used in this work were pure clonal lines, cultured from either single spores or single uredia. All were repeatedly tested for purity on the standard differential varieties of Mains and Jackson (*9*).

The following varieties of wheat were used in the infection and entry studies: Spring varieties, Hope (C. I.[3] 8178), Little Club (C. I. 4066), Marquis (C. I. 3641), Kota (C. I. 5878), Norka (C. I. 4377), Progress (C. I. 6902), and Webster (C. I. 3780); winter varieties, Michigan Amber selection 29–1–1–1, Gladden selection 455–1, and Kanred (C. I. 5146).

EXPERIMENTAL RESULTS AND OBSERVATIONS

RELATION OF LIGHT AND DARKNESS TO STOMATAL APERTURE

As shown by Loftfield (*8*) and confirmed in these studies, stomatal movements of the wheat plant in response to light are regular, the stomata opening only when the plants are illuminated and closing very soon in darkness. In the present study, direct observations throughout 24-hour periods were made upon the stomatal behavior of outdoor-grown seedlings of Gladden, Kota, Marquis, Michigan Amber, and Progress wheats kept continuously supplied with abundant moisture. These seedlings were subjected to complete darkness

[3] C. I. denotes accession number of the Division of Cereal Crops and Diseases.

and a saturated atmosphere at various times and for various periods of the day to ascertain whether the stomata ever opened in darkness under conditions of abundant soil moisture and high atmospheric humidity, which might be thought to favor such behavior. In those cases where the stomata were open when the plants were subjected to the above conditions they closed regularly within 15 minutes, although some reacted more promptly than others. Such closed stomata failed to open again in darkness, although those of the check plants in light maintained the characteristic daily response. If the stomata were closed when the plants entered the chambers they remained closed throughout their confinement in the dark. When the plants are held in darkness for 24 hours the stomata usually fail to open normally when first again exposed to light and may require considerable illumination before they resume their normal response to light. Certain stomata appear to be dead or nonfunctional. These have invariably been found to be closed.

The stomata of mature field plants of the varieties Harvest Queen, Michigan Amber, Leap, Kharkof, and Turkey were observed at 8:30 a. m. and 1 p. m. during the period from May 12 to 29, 1933, during which period there was abundant soil moisture. It was found, contrary to Loftfield's observations (*8*), that within a limited range all the stomata of a leaf reacted as a group, opening in like degree or closing simultaneously, according to environmental conditions, although on certain days little opening occurred.

Many other observations involving many inoculation trials have been made, but in no case have stomata been found to be open in plants kept in darkness, while in plants exposed to light in the usual manner the stomata have been found to open regularly on exposure to sunlight or to artificial illumination of sufficient intensity. The foregoing observations would seem to indicate that the stomata of the experimental plants remain closed throughout any period in which they are kept in darkness. Furthermore, it appears from microscopic observation of prepared epidermal strips and of the living leaf that when a stoma of a normal wheat plant closes there remains not even a small crevice between the guard cells through which a hypha of a rust fungus may enter without overcoming the pressure exerted by these cells. Thus, by determining the abundance of infection resulting from inoculations made under conditions of light and darkness and checking sample leaves for stomatal condition, it is possible to ascertain the relation of open and closed stomata to infection.

INFECTION FOLLOWING INOCULATION OF FIRST SEEDLING LEAVES HAVING OPEN OR CLOSED STOMATA

To determine whether the open or closed condition of stomata at the time of host entry by leaf rust has any effect on the abundance of infection, the following inoculations were made: The first seedling leaves of the winter wheats Gladden, Kanred, and Michigan Amber and of the spring wheats Kota, Marquis, and Progress were inoculated in the evening with each of the forms 9, 55, and 56. The plants were atomized with water and placed in a common dark humidity chamber until morning, when they were separated into two groups, one of which was placed in the lighted and the other in the dark

humidity chamber described above. The stomata in the lighted chamber were found to open in each trial, while those in the dark chamber remained closed. This simulated the condition of evening dew followed by morning opening of stomata in the light, as contrasted with continued closure of stomata. The plants were removed to the greenhouse bench the following evening.

With odds of 19 to 1 as the criterion of significance, there were only 15 out of a total of 36 comparisons in which a significant difference in number of pustules occurred (table 1). Of these 15 significant differences, 9 resulted in a greater infection in the lighted chambers and 6 in the dark chambers. Considering the fact that no open stomata have been observed on plants in the dark chambers, these data indicate no fundamental difference in the capacity of these forms of leaf rust to enter closed or open stomata of the six varieties of wheat studied. Such significant differences as were recorded appear to have been due to failure to distribute the inoculum uniformly over the experimental plants.

TABLE 1.—*Effect of open and closed stomata at time of stomatal entry on infection of first seedling leaf of 6 wheat varieties by Puccinia triticina, physiologic forms 9, 55, and 56*

Variety	Trial no.	Pustules,[1] under indicated stomatal condition, of physiologic form no.—								
		9			55			56		
		Stomata open	Stomata closed	Difference[2] ±S.E.	Stomata open	Stomata closed	Difference[2] ±S.E.	Stomata open	Stomata closed	Difference[2] ±S.E.
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Gladden	1	11.6	11.9	−0.3±1.64	30.6	31.2	−0.6±1.66	8.0	8.6	−0.6±0.64
	2	15.0	14.1	+.9±1.06	7.1	8.4	−1.3± .85	10.7	13.7	−3.0± .85
Kanred	1	11.0	9.1	+1.9±1.64	43.2	31.2	+12.0±1.66	7.6	6.2	+1.4± .64
	2	18.0	16.2	+1.8±1.06	8.9	7.6	+1.3± .85	6.2	3.7	+2.5± .85
Kota	1	7.4	5.9	+1.5±1.64	24.9	29.9	−5.0±1.66	4.0	4.6	−.6± .64
	2	22.1	21.3	+.8±1.06	7.8	6.7	+1.1± .85	6.1	4.9	+1.2± .85
Marquis	1	14.0	15.4	−1.4±1.64	23.4	30.0	−6.6±1.66	8.1	4.6	+3.5± .64
	2	9.0	12.8	−3.8±1.06	4.5	4.3	+.2± .85	8.3	7.8	+.5± .85
Michigan Amber	1	22.5	14.9	+7.6±1.64	25.1	28.7	−3.6±1.66	8.9	6.2	+2.7± .64
	2	10.8	9.6	+1.2±1.06	5.9	6.0	−.1± .85	7.5	8.0	−.5± .85
Progress	1	9.1	13.8	−4.7±1.64	30.9	16.1	+14.8±1.66	12.9	11.8	+1.1± .64
	2	10.5	9.2	+1.3±1.06	6.5	6.5	.0	6.5	5.0	+1.5± .85
Average		13.4	12.9	+.5± .56	18.2	17.2	+1.0± .53	7.9	7.1	+.8± .31

[1] Mean number per linear centimeter of leaf for 10 leaves.
[2] Open minus closed.

Since inoculations at 21° C. showed no effect of open or closed stomata on the abundance of infection, the question arises as to whether this is true at other temperatures. Therefore a group of spring wheat varieties was inoculated with form 9 under conditions of open and closed stomata as in the above experiments at the temperatures 15°, 22°, and 29°, controlled to ±1° (table 2). The trials at each temperature involved a single experiment. The abundance of infection was estimated on the scale of percentage of possible infection. Very little infection occurred at 29° with stomata either open or closed. Abundant infection took place at 15° and 22°, but there were no consistent differences in infection at either tempera-

ture between plants inoculated in the open or in the closed stomatal condition.

TABLE 2.—*Relation of temperature to infection by physiologic form 9 resulting from inoculations of first seedling leaf with stomata open and closed*

Variety	Infection under indicated stomata, condition at—					
	15° C.		22° C.		29° C.	
	Stomata open	Stomata closed	Stomata open	Stomata closed	Stomata open	Stomata closed
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Hope [1]	35	35				
Kota	45	25	35	60	Trace	Trace
Little Club	80	80	90	85	Trace	Trace
Webster	65	35	80	85	Trace	Trace
Norka [1]			75	90	Trace	Trace

[1] Hope was inoculated only at 15° and Norka only at 22° and 29°.

INFECTION BY SINGLE UREDIUM CULTURES THAT HAD BEEN CONTINUOUSLY PROPAGATED SINCE COLLECTION, BY INOCULATIONS IN LIGHT

The question arises as to whether all strains of *Puccinia triticina* occurring in nature are as capable of infecting when entry must occur through closed stomata as were the cultures of forms 9, 55, and 56 considered above. Since these three cultures had been propagated for several years without light during the period of stomatal entry, it seemed possible that they might have been unknowingly selected because of their ability to effect abundant entry in darkness. Therefore 15 single-uredium cultures, from collections made in 9 different States in 1932, which had been continuously propagated by inoculations in light from the time of collection, were tested for capacity to infect the first seedling leaf of Gladden, Michigan Amber, and Progress wheats having open or closed stomata during the period of stomatal entry. The conditions of open and closed stomata were brought about as in the above experiments. The results given in table 3 are the estimated percentages of infection. These cultures were found to comprise seven different physiologic forms and are fairly representative of those prevalent in the eastern part of the United States in 1932. There is no evidence that the initial open or closed condition of the stomata at the time of appressorium formation had any effect on the amount of infection by any of these cultures.

TABLE 3.—*Effect of open and closed stomata at time of stomatal entry on infection of first seedling leaf by 15 single-uredium cultures of Puccinia triticina that, after collection, had been continuously propagated by inoculation in light*

Place of collection	Physiologic form no.	Infection under indicated stomatal condition on—					
		Gladden		Michigan Amber		Progress	
		Stomata open	Stomata closed	Stomata open	Stomata closed	Stomata open	Stomata closed
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
East Lansing, Mich.	9	30	30	30	30	30	30
Marion County, Ind.	15	25	25	50	35	75	25
Elk Creek, Va.	15	15	15	15	15	15	25
Statesville, N. C.	15	60	60	50	50	75	75
Moultrie County, Ill.	39	65	75	65	75	65	65
Gainesville, Fla.	55	75	75	60	75	75	75
State College, Pa.	56	30	30	50	50	40	40
Columbus, Ohio	56	85	85	75	75	85	85
La Fayette, Ind.	56	75	75	75	75	90	50
Elkhart, Ind.	56	20	60	60	60	30	60
Vanderburgh County, Ind.	56	20	40	60	40	65	50
Raleigh, N. C.	56	50	50	50	50	50	50
Experiment, Ga.	60	50	50	50	50	50	50
Allen County, Ohio	61	10	15	35	35	50	40
Fairfield County, Ohio	61	15	15	50	15	45	35
Average		41.7	46.7	51.7	48.7	56.0	50.3

INFECTION RESULTS WITH MATURE PLANTS

In extensive testing of leaf rust resistance of mature wheat plants during the winter months in the greenhouse at La Fayette, Ind., heavy infection has usually been secured. The conditions during the period of possible fungus entry were such that it seemed reasonable to assume that entry took place through closed stomata. Thus, it has been the practice to shower the plants with inoculum at about 4:30 p. m., wet them with a fine spray of water, and cover with several layers of wet muslin cloth. The cloths were removed between 8 and 9 a. m. the next day and the plants allowed to dry at once. Since more than an hour of daylight is necessary to cause stomatal opening during the winter, it appears that the stomata usually remained closed until the moisture had evaporated from the plants. Further evidence that entry was effected before the plants were uncovered was obtained with a large series of mature plants in which abundant infection occurred, even though the wet plants were heavily dusted with sulphur for mildew control immediately following removal of the muslin cloths. Other experiments relating to the control of cereal rusts by the application of sulphur dust indicate that but little infection occurs after such treatment.

Mature Kota and Marquis wheat plants, in duplicate series of 15 plants each, were inoculated with physiologic form 56 and exposed to light and to darkness at 21° C. during the period of stomatal entry. Moderately abundant infection occurred from inoculations in the light, with slightly less from those in darkness—a result not unlike that obtained with seedlings and reported above.

FACILITY OF ENTRY THROUGH INITIALLY OPEN OR CLOSED STOMATA AS DETERMINED DIRECTLY BY SUBSTOMATAL VESICLE COUNTS

In the experiments described above, the ability of *Puccinia triticina* to enter the host stomata was inferred from the abundance of infection. This method is subject to inaccuracies incident to unequal distribution of both inoculum and moisture on the leaves. These defects were eliminated almost entirely by the technique described above, wherein the epidermis was stripped from the inoculated leaves and examined directly with the microscope to determine the percentage of appressoria that had effected entry.

Triplicate trials with physiologic form 55 on Michigan Amber were made by this method. The first leaves of seedling plants grown in the greenhouse were inoculated at 2:30 a. m. to insure abundant germination following the opening of the stomata. Some of the plants were placed in the dark and some in the lighted inoculation chamber. In the latter the stomata opened wide the next day and remained so until 4 p. m., when the epidermis was stripped. In the dark chamber the stomata remained closed all day. Unpublished data by the junior writer indicate that with physiologic form 55 stomatal entry occurs most abundantly between 6 and 12 hours after inoculation. It may therefore be assumed that in this experiment the majority of appressoria developed after the stomata had opened in the lighted chamber and over closed stomata in the dark chamber. By systematic inspection of the strips of epidermis the percentages of those appressoria from which entry had occurred were determined, and the results are presented in table 4. It will be seen that on leaves with open stomata an average of 89.8 percent of the appressoria had effected entry at the time of stripping, while, on leaves with closed stomata, 85.3 percent had succeeded. The difference seems insignificant in view of the high percentage of entries.

TABLE 4.—*Effect of open and closed stomata on stomatal entry by Puccinia triticina, physiologic form 55, in Michigan Amber wheat as determined by substomatal-vesicle counts*

Inoculation trial no.	Stomata open			Stomata closed		
	Appressoria not effecting entry	Appressoria effecting entry		Appressoria not effecting entry	Appressoria effecting entry	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
1	15	115	88.5	32	155	82.9
2	19	135	87.7	24	157	86.7
3	15	181	92.3	28	176	86.3
Total	49	431	89.8	84	488	85.3

MODE OF ENTRY AND ITS RELATION TO STOMATAL APERTURE

A study was made of the mode of stomatal entry into the first seedling leaf of plants of Gladden, Kanred, Kota, Marquis, Michigan Amber, and Progress wheats by 18 cultures of *Puccinia triticina*.

These cultures, collected from several different States, comprised the physiologic forms 9, 15, 39, 55, 56, 60, and 61. Epidermal strips, prepared as already described, were examined. Observations were made with respect to the entire sequence of development, from spore germination through the formation of the appressorium, the infecting hypha, and the first haustorium mother cell.

The formation of appressoria has been observed to occur exclusively over stomata. As shown by Allen (*2*), more than one appressorium may occupy a single stoma (fig. 1). Instead of producing a normal entry hypha, an occasional appressorium may develop a small vesicle on the surface of the leaf, similar in appearance to a substomatal vesicle. This, in turn, may produce a hypha similar in appearance to an infecting hypha, but on the leaf surface. The appressorium usually is formed upon one end of the stoma (fig. 2, *A*, *B*), but occa-

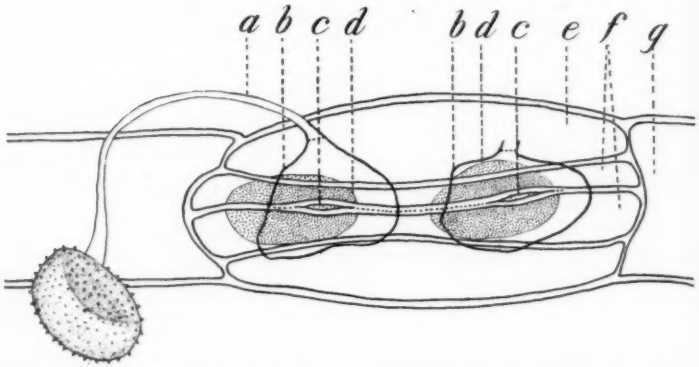

FIGURE 1.—Stoma of Michigan Amber wheat showing entry from two appressoria of *Puccinia triticina* physiologic form 55: *a*, Germ tube of urediospore; *b*, walls of empty appressoria; *c*, slits between guard cells forced open by entry of the rust; *d*, substomatal vesicles filled with protoplasm; *e*, accessory cell of stoma; *f*, guard cell of stoma; *g*, cell of epidermis. Drawing (semidiagrammatic) made with the aid of the camera lucida. × 875.

sionally it may be found near its center (fig. 3, *B*). Entrance never has been found to occur directly from a germ tube without the formation of an appressorium.

Although the appressorium may begin to form over an open stoma the latter soon closes, even before the appressorium is fully developed (fig. 2, *A*). Where appressoria form over closed stomata in darkness, the occupied stomata fail to open when the plants are again illuminated (fig. 2, *B*). In observations of hundreds of appressoria on strips of epidermis with normally open stomata, no instance has been found where a mature appressorium occupied a stoma that was more than very slightly open, and these latter cases were very rare.

Closure of stomata occupied by rust was shown to occur prior to entry in strips of epidermis of six wheat varieties inoculated with *Puccinia triticina* physiologic forms 55 and 56 (table 5). The strips were taken 6 to 8 hours after inoculation, before many entries had occurred and after exposure of the inoculated plants to light in the humidity chamber for from 3 to 4 hours. This was sufficient light to cause opening of a majority of the unoccupied stomata. On the epidermal strips the appressoria that have effected entry can be

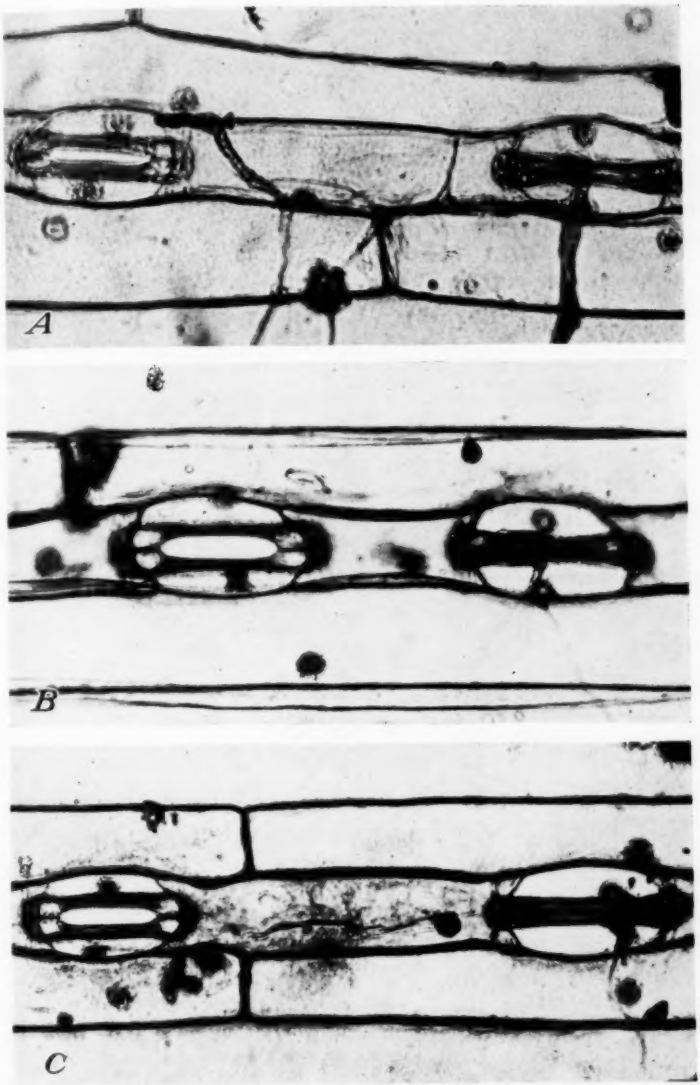

FIGURE 2.—*A*, At right, photomicrograph of an immature appressorium of *Puccinia triticina* physiologic form 9 over a closed stoma of the first seedling leaf of a Michigan Amber wheat plant. This appressorium probably began to form while the stoma was yet open. At left, an open, unoccupied stoma. × about 395. *B*, At right, similar to *A*, except that the appressorium is mature and probably was formed at night over a closed stoma which failed to open in the morning. Entry has not yet occurred. × about 395. At left, an open, unoccupied stoma. *C*, At right, similar to *A*, but with *Uromyces trifolii*. × about 395. At left, an open, unoccupied stoma.

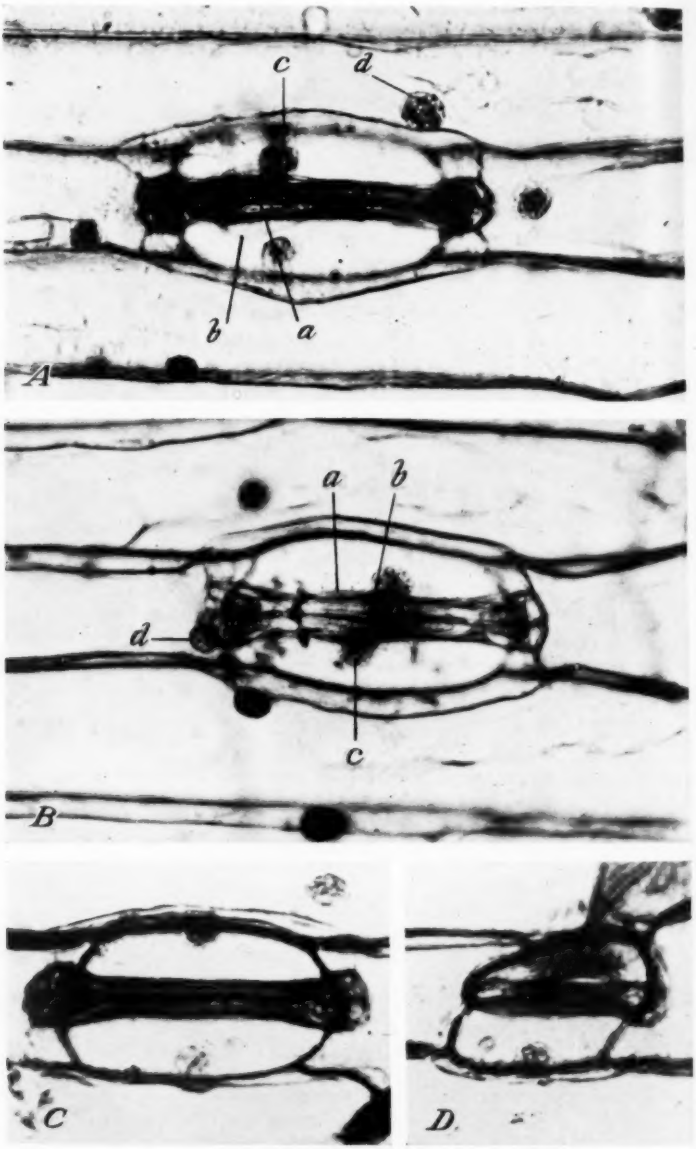

FIGURE 3.—For explanatory legend see opposite page.

distinguished readily from those that have not by the empty appressorium wall and by the dark-staining substomatal vesicle (fig. 4, *A*). The stomata of all six varieties apparently reacted alike to the appressoria in closing prior to entry by the fungus. Of a total of 618 observations of individual appressoria of physiologic form 55, 615 had caused the underlying stomata to close, while 3 had permitted a very narrow slit to persist. Of the unoccupied stomata observed on the same epidermal strips, 94 percent were open. The behavior of the six wheat varieties was almost identical. Similar results were obtained from physiologic form 56.

TABLE 5.—*Effect of appressoria of Puccinia triticina physiologic forms 55 and 56, prior to entry, on stomatal function of first seedling leaf of wheat* [1]

Variety	Inoculated with *P. triticina* physiologic form 55						Inoculated with *P. triticina* physiologic form 56					
	Occupied stomata			Unoccupied stomata			Occupied stomata			Unoccupied stomata		
	Closed	Open		Closed	Open		Closed	Open		Closed	Open	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Michigan Amber	118	0	0	28	472	94.4	164	0	0	18	682	97.4
Gladden	62	2	3.13	65	838	92.8	66	1	1.49	44	466	91.4
Kanred	75	0	0	42	658	94.0	83	0	0	93	707	88.4
Kota	126	1	.79	42	858	95.3	45	0	0	11	389	97.3
Marquis	86	0	0	49	651	93.0	28	0	0	13	387	96.8
Progress	148	0	0	58	942	94.2	32	0	0	22	400	94.8
Total	615	3	.49	284	4,419	94.0	418	1	.24	201	3,031	93.8

[1] Plants were held in illuminated humidity chambers following inoculation until the time of stripping.

Following stomatal entry and subsequent transfer of the protoplasm from the appressorium to the substomatal vesicles or infecting hyphae, the stomata still fail to open when exposed to light. In table 6 data are given from Michigan Amber plants, inoculated with physiologic form 55, from which the epidermis was stripped about 18 hours after inoculation and after unoccupied stomata had been induced to open in the light. No attempt was made to determine whether entry had been effected from each appressorium observed, but it was obvious that all but a very few appressoria were empty. Of 536 stomata occupied by appressoria and presumably entered, 535 were tightly closed while 1 possessed a narrow slit. Of 1,641 unoccupied stomata on the same strips of epidermis, 86.6 percent were open.

EXPLANATORY LEGEND FOR FIGURE 3

A, Photomicrograph of a stoma of the seedling leaf of Michigan Amber wheat entered by *Puccinia triticina* physiologic form 9 near one end of the stoma. *a*, Stomatal slit opened by the fungus; *b*, wall of empty appressorium; *c* and *d*, nuclei of an accessory cell and epidermal cell, respectively, that have become oriented near the point of entry. × about 707. *B*, Similar to *A*, with physiologic form 56, but entry occurred at the approximate center of the stoma. *a*, Wall of empty appressorium; *b*, stomatal slit opened by the fungus; *c* and *d*, nuclei of an accessory and epidermal cell, respectively, that have become oriented near the point of entry. × about 707. *C*, A normal stoma of Michigan Amber closed in darkness; no opening present. × about 707. *D*, A minute slit in an abnormal stoma, adjacent to that in *C*, in which the accessory cell has been replaced by a hair cell, preventing the normal closing of the stoma. × about 707.

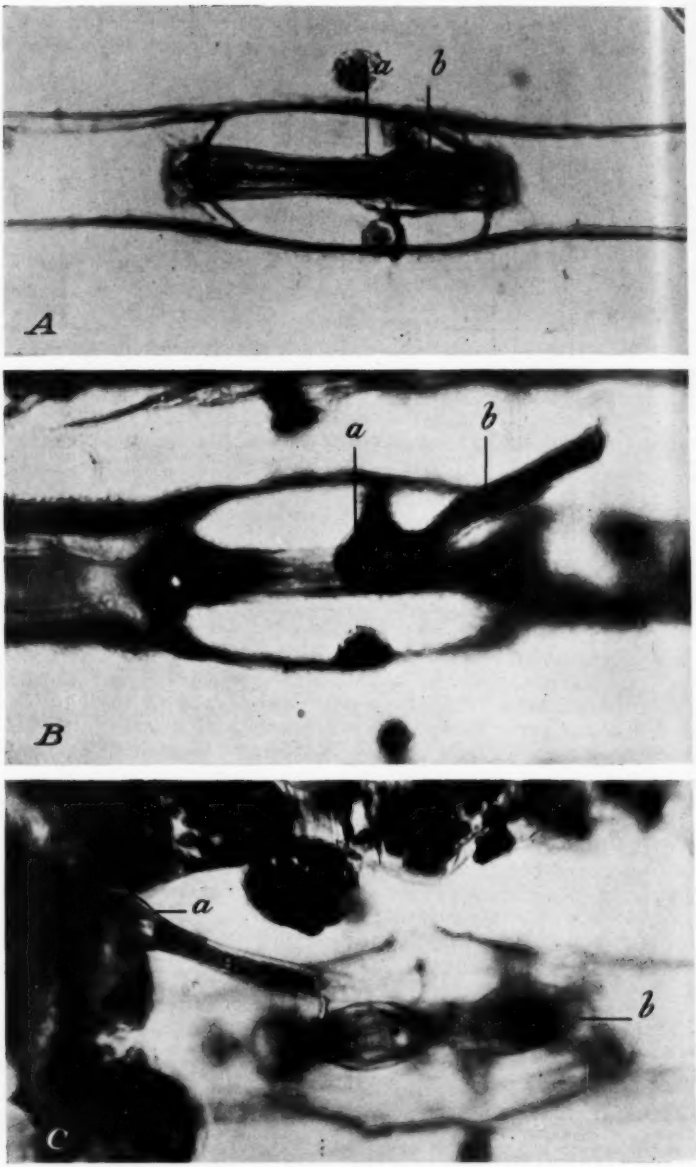

FIGURE 4.—For explanatory legend see opposite page.

TABLE 6.—*Effect of Puccinia triticina physiologic form 55, subsequent to entry, on stomatal function of first seedling leaf of Michigan Amber wheat*

Inoculation trial no.	Stomata entered			Stomata unentered		
	Stomata closed	Stomata open		Stomata closed	Stomata open	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
1	252	1	0.4	180	626	77.7
2	154	0	0	18	330	94.8
3	129	0	0	22	465	95.5
Total	535	1	.2	220	1,421	86.6

The direct observation of the effect of the living appressoria on stomata of living plants was facilitated by the use of a Leitz Ultropaque microscope. For this work wheat seedlings were grown in small glass vials and the whole was mounted on the mechanical stage of the microscope. The seedling leaves were inoculated with leaf rust and were examined after the urediospores had germinated, and the unoccupied stomata were induced to open in light. Many stomata occupied by appressoria were observed and all were completely closed, while neighboring unoccupied stomata were very largely open.

The entry hypha from the appressorium apparently exerts a pressure in pushing through between the closed guard cells. In many cases where the stomata were favorably oriented and the contents of the appressoria had moved into the substomatal vesicles or infecting hyphae, it was possible to see clearly, through the transparent appressorium, the minute lens-shaped opening between the guard cells, apparently produced by the force of the entry hypha (figs. 1 and 3, *A*, *B*). The entries shown in figures 1 and 3, *A*, are at the usual point of entry, i. e., at the ends of the stoma. Figure 3, *B*, however, shows a case where entry was effected at the center of the stoma.

The study of strips of epidermis fixed in alcohol and of the living leaf by means of the Leitz Ultropaque microscope failed to disclose in normally closed stomata any openings such as are shown below the appressoria in figures 1 and 3, *A*, *B*. Were such minute slits normally present they would be readily observed in alcohol mounts of epidermis, as is shown in figure 3, *D*. Here the accessory cell has failed to develop and has been replaced by a hair cell which inter-

EXPLANATORY LEGEND FOR FIGURE 4

A, Photomicrograph from the outside of a stoma of a seedling leaf of Michigan Amber wheat. The stoma has been invaded by an entry hypha of *Puccinia triticina* physiologic form 9. Taken with a low-power objective to give depth of focus, showing the empty appressorium wall at *a*, and the deep-staining substomatal vesicle at *b*. × about 707. *B*, As in *A*, with physiologic form 39, but viewed from the inner side of the epidermis strip. *a*, Protoplasm-filled substomatal vesicle; *b*, young infecting hypha. × about 790. *C*, Photomicrograph of two substomatal vesicles and one infecting hypha of *P. triticina* physiologic form 15 viewed from the inner side of the epidermis of the first leaf of a Gladden wheat plant. *a*, A haustorium mother cell wedged in place against the mesophyll cells. Note that the infecting hypha grew out parallel to the epidermis. A mesophyll cell showing as a shadow over the haustorium mother cell, because above the focal plane, makes relatively certain the conclusion that the haustorium mother cell was undisturbed in the making of the preparation. *b*, Empty substomatal vesicle from which infecting hypha was produced. A portion of the infecting hypha is out of focus and shows as a shadow over the vesicle at the left. × about 707.

fered with the normal functioning of the stoma. Other stomata on this specimen of epidermis were tightly closed, as shown in figure 3, *C*, from a photomicrograph of the stoma nearest to that shown in figure 3, *D*.

There commonly is an orientation of the nuclei of the stomatal accessory cells and adjacent epidermal cells to the wall nearest to a point of entry, suggesting a response to an injury or a chemical stimulus (figs. 3, *A*, *B*, and 4, *A*). Normally, for noninvaded stomata, the nuclei of the accessory cells lie against the wall outermost from the guard cells (fig. 3, *C*, *D*).

One infecting hypha was regularly produced, as found by Allen (*3*). An entire substomatal vesicle and an infecting hypha are shown in figure 4, *B*. In figure 4, *C*, is shown a haustorium mother cell wedged in the angle between mesophyll cells that have adhered to the epidermis. In this and other instances the infecting hyphae quite definitely grew out approximately parallel to the epidermis rather than at right angles, which, as Allen (*1*) has indicated, was characteristic for the culture of *Puccinia triticina* form 11 with which she worked. These cases may have been exceptions, since infecting hyphae extending directly into the substomatal cavity would very probably be disturbed in the preparation of the epidermal strips.

A study was made of the entry of *Uromyces trifolii* (Hedw. f.) Lév. from red clover (*Trifolium pratense* L.) into the first seedling leaf of Michigan Amber wheat. In two such inoculation experiments, reported in table 7, the behavior of this rust on wheat was identical with that of *Puccinia triticina* in readily entering the stomata, which regularly closed prior to entry (fig. 1, *c*).

TABLE 7.—*Effect of appressoria of clover rust (Uromyces trifolii), prior to entry, on stomatal function of first seedling leaf of Michigan Amber wheat*

Inoculation trial no.	Occupied stomata			Unoccupied stomata		
	Closed	Open		Closed	Open	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
1	241	0	0	29	180	86.1
2	193	0	0	17	243	93.5

DISCUSSION

While it is not possible to generalize fully on the capacity of the leaf rust of wheat to enter closed stomata, this study indicates that it is unlikely that any of the strains of this rust occurring in the United States depend upon open stomata for host invasions. Hart (*6*) has described a type of resistance to stem rust (*Puccinia graminis tritici*), which she terms "functional resistance." This consists of a habit of delayed morning opening of stomata of certain wheats, preventing the entry of this rust until the moisture on the plant has evaporated, thus exposing the delicate germ tubes to desiccation and death before entry can be effected. The results of the present study would seem to dismiss the possibility of this type of resistance to leaf rust, since each of the 18 cultures of leaf rust studied was able to enter closed stomata and since forms 9, 55, and 56, studied in greater detail,

apparently never enter open stomata because of the prompt stomatal closure in response to appressorium formation.

It has been generally assumed (*2, 6, 8*) that entry of the cereal host by rusts occurs only through open stomata. The appressorium of the germinating urediospore has apparently been considered to have no function beyond that of a germ tube. So far as concerns the cultures of leaf rust here employed, it is highly probable that the appressorium is an organ particularly adapted to force stomatal entry, having a function somewhat similar to that of the appressorium originating from the basidiospore, which effects direct penetration of the epidermis.

Allen (*1, 2*) states that the appressorium probably yields some toxic secretion that exerts a harmful effect on the host cells, in some instances even killing the guard cells. Whatever the nature of this injury, it does not appear to be the sole means by which entry through closed stomata is made possible, although it may play a part in that process. The very small stomatal slit forced open by the entry hypha indicates that a definitely localized pressure is exerted on the stoma, just as happens in cases of direct penetration of the epidermis of the alternate hosts by the gametophytic phase of rusts and by other fungi effecting direct penetration.

The response of the nuclei of the stomatal accessory and epidermal cells of the host in orienting themselves against the cell walls nearest to an entering leaf rust fungus supplements Allen's observation (*2, 3*) on the effects of the leaf rust fungus on the position of nuclei of accessory cells and the condition of the entire protoplast of the guard cells. She noted in Malakof wheat (resistant) at least two instances in which the accessory cell nuclei lay as close as possible to the entering fungus, while in Little Club (susceptible) the staining reaction indicated that the protoplasts of the guard cells of the invaded stomata often were killed and their walls altered chemically. She (*1*) further observed in the case of stem rust, *Puccinia graminis tritici*, that the guard cells and adjoining epidermal cells of both susceptible and resistant varieties may be severely injured or killed by the effect of the appressorium. The orientation of the nuclei observed in the writers' studies suggests a traumatotactic response similar to that of corn seedlings injured by disease or otherwise, as reported by Pearson (*10*) and in other investigations reviewed by her. In the case of leaf rust, where the invasion of the cereal host occurs through a forced entry of stomata, it is possible that this injury may be a means of facilitating such entry. On the other hand, the injury may be quite unrelated to entry.

SUMMARY AND CONCLUSIONS

A simple technique is described for studying the entrance phenomena of a rust in relation to stomatal aperture. This involves the stripping of the inoculated epidermis from the mesophyll cells and its fixation in absolute alcohol containing a suitable stain.

The initial open condition of stomata of seedling wheat, prior to appressorium formation, is unnecessary to entry by leaf rust (*Puccinia triticina*), as represented by 18 cultures comprising 7 physiologic forms. Limited studies suggest the same relation for mature plants.

The normally closed stomata of wheat seedlings provide no openings through which entry hyphae of leaf rust can pass without overcoming the resistance exerted by the tightly closed guard cells. When an appressorium of leaf rust is initiated on an open stoma of a seedling leaf, the stoma closes tightly prior to entry.

The appressorium originating from the urediospore appears to function as a specialized organ to apply pressure between the closed guard cells, thereby effecting a forced opening of the stoma and entry by the fungus.

Since closed stomata offer no effective barrier to the entry of *Puccinia triticina*, there is no possibility of "functional resistance" to this rust through delayed morning opening of stomata.

Appressoria of clover rust (*Uromyces trifolii*) also cause wheat stomata to close and have been observed to effect numerous entries through initially closed stomata of seedling wheat leaves.

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INHIBITING EFFECT OF SULPHUR IN SELENIZED SOIL ON TOXICITY OF WHEAT TO RATS[1]

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INTRODUCTION

The recent discovery (*9*, *13*)[3] that a serious disease of livestock is caused by ingestion of selenium contained in vegetation grown on certain soils of the Middle Western States led to an investigation of the absorption of selenium by plants and of the factors affecting it (*10*, *11*). In the course of these investigations it was found that the addition of but 1 part per million of selenium as sodium selenate to the soil (Keyport clay loam) rendered wheat grown thereon toxic to white rats (*14*). Later it was found[4] that wheat grown with this concentration of selenium produced symptoms of injury similar to but not so extreme as those produced by feeding the naturally toxic wheat from the Middle West. There soon followed the discovery that the quantity of selenium absorbed by the plants could be reduced by increasing the sulphur in the soil or nutrient solution (*10*, *11*). The bearing of this interrelationship of the two elements on the problem of control or amelioration of the disease in seleniferous soil areas was at once apparent. Experiments were therefore designed to determine whether the addition of excess sulphur to a selenized soil would reduce or prevent the toxicity of wheat grown upon it. The results of these experiments are reported in the present paper.

METHODS

A winter wheat (Dixie, a strain of Purplestraw) was grown at the Arlington Experiment Farm, Rosslyn, Va., on eight outdoor plots of Keyport clay loam about 5 feet square separated by 8-inch buffer zones of soil between boards extending 6 inches into the ground. To six of these plots selenium was added at the rate of 2 p. p. m. as sodium selenate ($Na_2SeO_4.10\ H_2O$), calculated on the basis of a depth of 6 inches of soil (5 g sodium selenate in 1,200 pounds of soil). Two of the plots, nos. 2 and 6, received only the selenium. Plots 3 and 7

[1] Received for publication Feb. 1, 1936; issued July 1936.

[2] The writers are indebted to Hazel E. Munsell, in charge of the Foods and Nutrition Division of the Bureau of Home Economics, for general supervision of the feeding tests.

[3] Reference is made by number (italic) to Literature Cited, p. 941.

[4] The toxicity of this grain, grown in quartz sand cultures, has been studied in comparison with that of the naturally toxic grain from South Dakota by Hazel E. Munsell, Grace M. De Vaney, and Mary H. Kennedy, of the Bureau of Home Economics, and their report is being prepared for publication.

received in addition flowers of sulphur at the rate of 1,500 pounds per acre (1 pound per plot), and plots 4 and 8 received the same amount of sulphur in the form of gypsum (5 pounds per plot). Two control plots, nos. 1 and 5, received no treatment.

The sulphur and gypsum ($CaSO_4$) were added first and thoroughly mixed with the soil to a depth of 6 inches. The requisite amount of sodium selenate (5.08 g, supplying 1.09 g of selenium) was added 2 days later. It was dissolved in several gallons of water in a watering can and carefully sprinkled on the soil, which was then thoroughly turned and mixed to a depth of 6 inches. The seed was sown on October 18, 1933. Some of the young plants were cut the following spring (April 18, 1934) for selenium analyses, and the rest were allowed to mature. Neither the plants nor the grain from the selenized plots differed in appearance from those of the control plots receiving no selenium, 2 p. p. m. selenium in this soil being insufficient to produce symptoms of injury (*10*, *11*).

White rats were used to test the toxicity of the grain, since they clearly indicate the presence of small amounts of selenium in their food by an initial reluctance to eat and by pronounced tissue changes in the liver (*5*, *8*). The grain was incorporated in the diet of the young rats when they were weaned at the age of 28 days. The grain constituted 70 percent of the diet, the rest being skim-milk powder (13 percent), dried bone and meat scrap (5 percent), yeast powder (5 percent), butter (5 percent), and cod-liver oil (2 percent). All the rats of a given experiment were of the same sex and from the same litter and were kept in individual cages and weighed weekly.

The feeding tests comprised three experiments. In the first, four female rats were fed all they would eat of the diets containing the grain from plots 1 to 4, respectively. The second experiment was a duplication of the first, with grain from plots 5 to 8 fed similarly to female rats of another litter.

Since the more selenium there is in the diet the less the rats will eat (*5*, *8*), a third experiment was designed to distinguish between the direct effects of the selenium and the effects due to inanition from the reduced food intake. Litter-mate males were fed grain from plots 1, 3, and 4 limited in amount to that eaten during the preceding 24 hours by a rat fed the "selenium-alone" grain from plot 2. In order to determine the extent to which inanition from insufficient food supply determined the growth rates of these rats, a fifth male of the litter was fed all he would eat of the control grain from plot 1.

At the close of the experiments the rats were autopsied, and the livers especially were examined for evidence of selenium poisoning.

RESULTS

The average weekly food consumption of the rats, their initial and final weights, and the presence or absence of liver symptoms at autopsy are given in table 1. The growth curves are presented in figures 1 and 2.

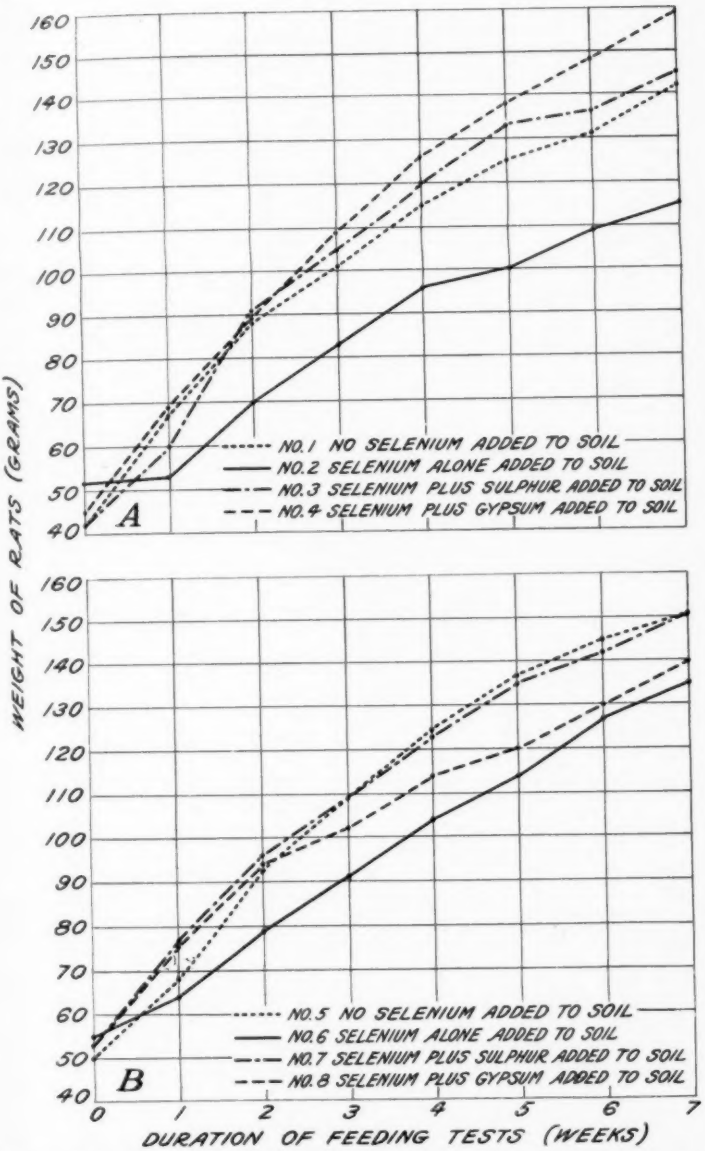

FIGURE 1.—Growth curves of female rats fed all they would eat of diets containing grain from untreated soil and from soil treated with 2 p. p. m. of selenium (as sodium selenate), with and without sulphur applications: *A*, First experiment, with grain from plots 1 to 4; *B*, second experiment, with grain from the duplicate plots 5 to 8. The feeding of the second group was continued longer than that of the first, and the final weights, not included in this graph, are given in table 1.

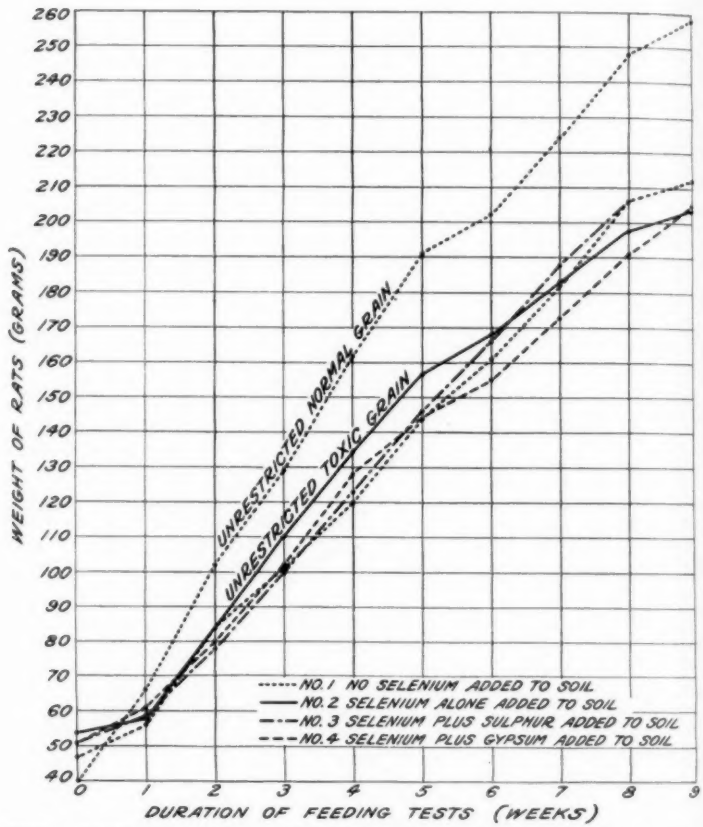

FIGURE 2.—Growth curves of male rats of the third experiment, fed diets containing grain from plots 1 to 4, with the daily food intake of rats 1, 3, and 4 limited to that of the rat fed on the toxic grain from plot 2 (curve marked "unrestricted toxic grain"). The curve marked "unrestricted normal grain" is that of a male rat (no. 5) from the same litter fed all he would eat of the diet containing control grain from plot 1.

TABLE 1.—*Effect on white rats of feeding wheat grown on selenized soil with and without applications of sulphur*

Experiment and rat no.	Soil plot no.	Soil treatment: Selenium added in parts per million	Soil treatment: Form of sulphur added	Average weekly food intake of rats (in grams) during—: First week	Second to fourth weeks	Fifth to seventh weeks	Eighth to ninth weeks	Tenth to eleventh weeks	Growth of rats (in grams): Initial weight	Final weight	Gain	Presence (+) or absence (−) of symptoms of selenium poisoning in the liver
1. Unrestricted feeding:												
Rat no. 1	1	0	None	49.4	62.2	69.4	.....	.....	42	142	100	−
Rat no. 2	2	2	do	22.6	44.1	46.6	.....	.....	52	115	63	+
Rat no. 3	3	2	Sulphur	40.0	68.0	63.7	.....	.....	42	145	103	−
Rat no. 4	4	2	Gypsum	45.1	62.9	66.4	.....	.....	45	159	114	−
2. Unrestricted feeding:												
Rat no. 1	5	0	None	60.0	70.4	67.6	69.2	68.5	50	177	127	−
Rat no. 2	6	2	do	42.8	49.6	58.2	61.2	62.4	55	162	107	+
Rat no. 3	7	2	Sulphur	59.6	61.0	63.5	68.7	69.3	53	179	126	−
Rat no. 4	8	2	Gypsum	55.0	56.8	57.5	58.7	55.8	53	157	104	−
3. Restricted feeding: [1]												
Rat no. 1	1	0	None	36.5	62.4	72.2	72.4	.....	47	212	165	−
Rat no. 2	2	2	do	36.2	64.6	78.7	73.3	.....	54	204	150	+
Rat no. 3	3	2	Sulphur	36.5	62.4	78.8	[2]	.....	51	[2]	.....	−
Rat no. 4	4	2	Gypsum	36.5	62.4	65.0	68.4	.....	51	205	154	−
Rat no. 5	1	0	None	47.9	80.3	87.0	90.9	.....	40	258	218	−

[1] Feeding of rats 1, 3, and 4 restricted to that of no. 2.
[2] Owing to exhaustion of the "selenium-plus-sulphur" grain sample, this rat was killed and autopsied a week before the others, at which time its weight was the same as that of the control on normal grain, i. e., 206 g.

During the first week of the unrestricted feeding experiments the rats on the grain samples from plots 2 and 6, treated with selenium alone, ate much less and grew far more slowly than those fed grain from the similarly selenized plots that had received sulphur or gypsum in addition (fig. 1, *A* and *B*). After the first week the difference became less pronounced as the rats apparently became used to the abnormal smell or taste of the "selenium-alone" grain and ate more normally, with a corresponding increase in rate of growth. Since marked initial retardation of growth is characteristic of animals given food containing selenium (*5*, *6*, *8*), the comparative growth rates during the first week of feeding showed clearly that the sulphur and gypsum treatments of the soil had so reduced the selenium intake of the plants that the grain did not retard the growth of the rats.

In the third experiment the growth rates of the rats on diets containing grain from plots 1, 3, and 4, respectively, each restricted in amount to that eaten by the rat on grain from plot 2, were all much the same (fig. 2), indicating that the initial retardation in the growth of the rats on grain from the unsulphured selenized plots 2 and 6 was largely attributable to disinclination to eat rather than to a specific physiological effect of the ingested selenium. Since the rat on the grain from plot 2 was fed all that he would eat, comparison of his growth curve with that of the fifth male of the same litter on an unrestricted diet of normal grain shows the extent to which the former's growth was hindered (fig. 2). The 2 p. p. m. selenium added to the soil did not make the grain sufficiently toxic to kill the rats in any of the experiments.

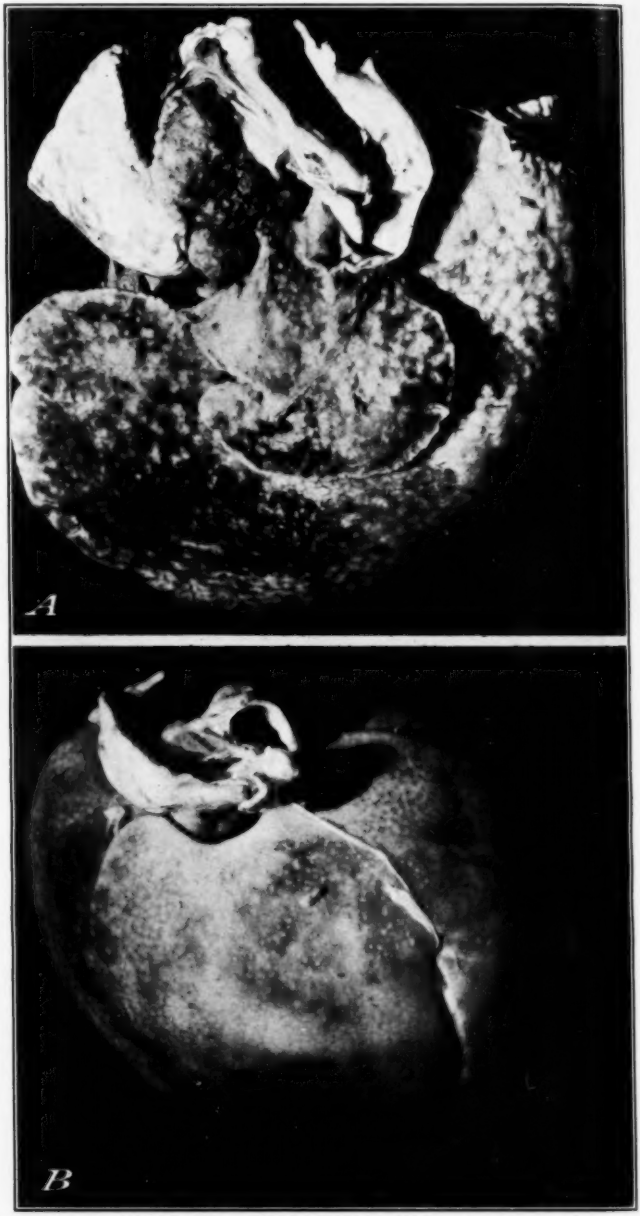

FIGURE 3.—*A*, Liver of rat of second experiment injured by selenium in grain grown on Keyport clay loam to which was added 2 p. p. m. of selenium as sodium selenate; *B*, normal liver. × 2.

The evidence from the growth curves that the grain from the selenized soil treated with sulphur or gypsum was less toxic than that from the plots receiving selenium alone was corroborated by the autopsy findings. Liver injury is the most obvious effect of mild selenium poisoning in rats (*5*, *6*, *8*). In the present experiments such injury characterized all the rats on grain from the unsulphured selenized soils. The upper lobe was small and atrophied; the one immediately beneath was enlarged and thickened (fig. 3). In one case the lobes were held together by adhesions. The surfaces were roughened by prominent lobules, which produced a granular appearance rather than the extreme nodulose distortion accompanying more severe poisoning (*5*). In a photograph made with light from a mercury lamp (fig. 4, *C*) the liver had a blanched appearance suggestive of the anemic condition characteristic of selenized rats (*5*, *7*, *12*). In marked contrast, the livers of the rats fed grain from the selenized soil treated with sulphur or gypsum appeared normal (fig. 4, *D* and *E*), like those of the rats fed control grain from the unselenized plots (fig. 4, *A* and *B*). The livers therefore showed very convincingly that the excess sulphur added to the selenized soil had prevented the grain from absorbing toxic concentrations of selenium.

On analysis, the grain that produced the selenium-damaged liver shown in figure 4, *C*, was found to contain about 12 p. p. m. of selenium (making a concentration of 8.4 p. p. m. in the diet). The nontoxic grain from the similarly selenized plots given sulphur or gypsum applications contained about 4 p. p. m. (making 2.8 p. p. m. in the diet).

TABLE 2.—*Analyses of plant material from the plots producing the grain of the feeding tests*

Soil plot no.	Soil treatment		Effect of first year's grain on rats	Selenium in plants and grain—					
				First year				Second year, grain [2]	
	Selenium added	Form of sulphur added		Young plants [1]	Mature plants [2]	Grain [3]			
	P. p. m.			*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	0	None	Nontoxic	0	0.5			0.0	0.0
2	2	do	Toxic	110	12.0			2.0	1.0
3	2	Sulphur	Nontoxic	2	3.0	3		.3	.1
4	2	Gypsum	do	8	3.0	5	4	.1	.2
5	0	None	do	0					
6	2	do	Toxic	50	13.0	12	11	5.0	6.0
7	2	Sulphur	Nontoxic	2	7.0	4	3	.4	.3
8	2	Gypsum	do	5	3.0	4	4	.6	.5

[1] Gravimetric determinations by A. Van Kleeck under the direction of H. G. Byers, of the Bureau of Chemistry and Soils.
[2] Gravimetric determinations by R. F. Gardiner and R. B. Deemer under the direction of P. R. Dawson, of the Bureau of Plant Industry.
[3] Colorimetric determinations in duplicate by L. H. Greathouse, of the Bureau of Plant Industry.

The analyses of grain from the various selenized plots are summarized in table 2 (except that from plot 2, which was entirely consumed in the feeding tests), together with analyses of the plants themselves at an early stage of development (tillering) and at maturity. Analyses of grain from the same plots the following year (1935) with no additional treatments of the soil are also included. The data all give evidence of reduced selenium absorption in the presence of

excess sulphur, the reduction being even more pronounced the second year than the first.[5]

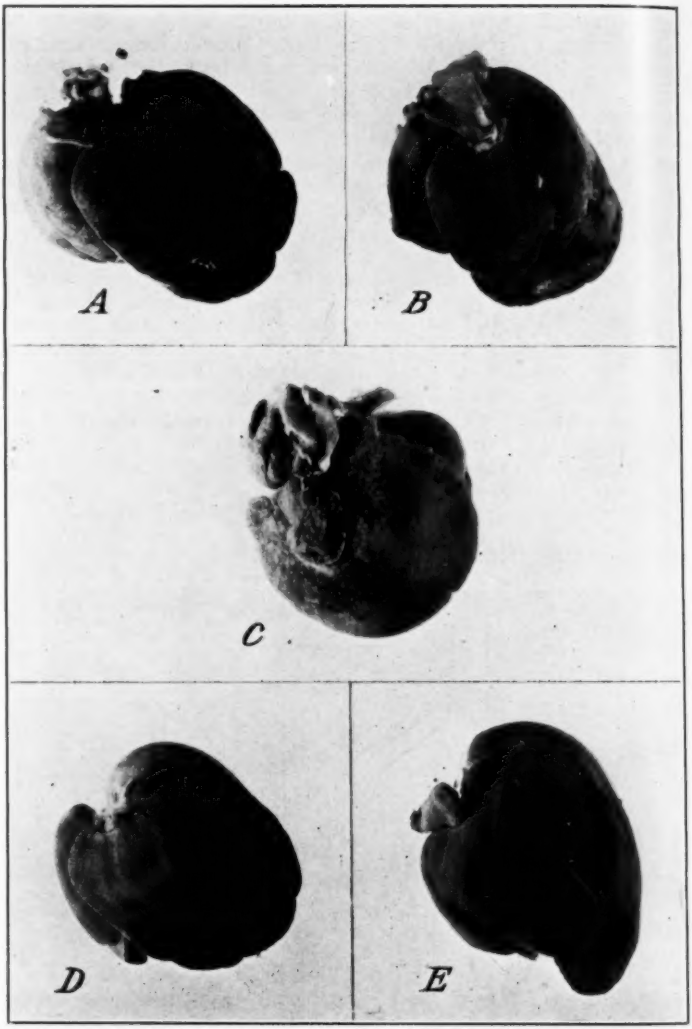

FIGURE 4.—Livers of rats fed: (*A* and *B*) Control grain from plot 5; (*C*) grain from plot 6 to which 2 p. p. m. of selenium as sodium selenate was added; (*D*) grain from plot 8 receiving the same quantity of selenium plus gypsum; (*E*) grain from plot 7 receiving the selenium plus sulphur. All five livers were photographed on one plate with light from a mercury lamp. Natural size.

[5] A third year's analyses of young plants from these plots showed that but a trace of available selenium remained in the soil. The plants from the selenized plots 2 and 6 without sulphur or gypsum still contained the most selenium, namely, 0.6 and 0.7 p. p. m. — where 50 to 110 p. p. m. had been found 2 years before. Evidence of a rapid decrease in selenium absorption by successive crops on the same soil appears also in the grain analyses for the first and second years.

That grain grown on soil treated with but 2 p. p. m. of selenium (5 g of sodium selenate in 1,200 pounds of soil) should be toxic emphasizes the seriousness of the problem in areas where the soil contains even higher concentrations (*3*). The problem exists because of the capacity of the plant for accumulating large quantities of selenium from small amounts in the soil, a capacity possessed in extraordinary degree by certain forage plants of the Middle West where the selenium disease of livestock occurs (*1, 2, 3, 4, 9*). Whether sulphur treatments will prove fully effective and feasible for reducing the absorption of selenium by vegetation in these areas remains to be determined.

SUMMARY

In the present experiments wheat grown on soil containing 2 p. p. m. of selenium and comprising 70 percent of the diet of white rats produced the retarded growth and the liver injury characteristic of selenium poisoning. Wheat grown on similarly selenized soil treated with flowers of sulphur or with gypsum was not toxic. Chemical analysis of the grain showed that the sulphur and gypsum treatments reduced the concentration of selenium in the grain from about 12 p. p. m. to about 4 p. p. m.

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RESPONSE OF CERTAIN PLANTS TO LENGTH OF DAY AND TEMPERATURE UNDER CONTROLLED CONDITIONS[1]

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INTRODUCTION

Since the appearance of the first paper of Garner and Allard on photoperiodism (*5*)[2] an extensive literature covering various phases of the subject has developed. The accumulated data as a whole are sufficiently comprehensive and consistent to remove any doubt as to the practical importance of length of day as a factor in plant growth and development. However, only in comparatively few instances have the experiments in this field been conducted with sufficiently accurate control of the environment as a whole to yield results of a quantitative nature that could be readily reproduced. Since in most instances natural illumination, with or without supplemental artificial light, has been employed, control of the illumination conditions other than daily duration has been out of the question, and usually only partial control of other environmental factors has been attempted.

Inasmuch as growth and development within the limits imposed by heredity ordinarily are the net result of interaction of the many environmental factors influencing the plant, it is necessary to supplement the above-mentioned data with observations made under controlled conditions and with suitable modifications of factors other than light duration if accurate information is to be obtained on the relationship of other factors to the day-length effect. It is true that rigid control of the environment necessarily will introduce conditions differing more or less from those obtaining in nature, but insofar as normal growth and development of the plant can be obtained, this method appears to furnish the best available means for studying the interrelation of the light period and other factors.

In the present paper results obtained in growing typical short-day and long-day plants under controlled conditions are presented. The primary objectives were (1) to compare plant response to the daily light period under rigidly fixed environments involving artificial illumination with results previously obtained under more or less natural conditions and (2) to make a direct study of the interrelation of day length and temperature as ecological factors. To culture the higher plants under adequately controlled conditions offers many experimental difficulties and at best is both laborious and expensive, so the data presented are necessarily limited. These data, however, appear to throw additional light on the photoperiodic responses of the plants studied and on the relation of temperature to these responses. With the artificial climates employed it has been possible to obtain photoperiodic responses closely agreeing with those observed under outdoor conditions.

[1] Received for publication Jan. 23, 1936; issued July 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 959.

MATERIAL AND METHODS

Soybeans (*Soja max* (L.) Piper), sugar beets (*Beta vulgaris* L.), and *Rudbeckia bicolor* Nutt. were used in the experiments, all of which were concerned mainly with initiation of sexual reproduction. Special attention was given to the comparative responses of the Mandarin, Peking, and Biloxi varieties of soybean, which in the latitude of Washington, D. C., behave as early, medium, and late sorts, respectively. The seed used were of strains supplied by the Division of Forage Crops and Diseases. At Washington the Biloxi and Peking varieties are markedly delayed in flowering by exposure to the maximum day length of summer, amounting to nearly 15 hours, and clearly are to be classed as short-day types. Although the 15-hour day has only a very slight delaying action on time of flowering of the Mandarin, it will be shown in the present paper that this variety differs essentially from the earlier ones in its photoperiodic response only in that its critical light period—that is, the maximum day length at which flowering ordinarily takes place—is considerably in excess of the 15-hour maximum length of day at Washington. There appears to be no reason, therefore, why this plant should not be regarded as a short-day type (*4*). The beet seed used were of a selected strain furnished by the Division of Sugar Plant Investigations. Commercial seed of *Rudbeckia* were employed. The beet and *Rudbeckia* are long-day types.

The apparatus used for control of environment has been described by Steinberg (*10*). It consists essentially of eight small light-proof cabinets through which conditioned air is circulated at a definite rate and which are provided with lighting units designed to furnish high-intensity illumination from 1,000-watt gas-filled tungsten lamps without material disturbance of the temperature of the air about the plants. In the experiments with soybeans the old type of lighting equipment was used, consisting of the ordinary pear-shaped 1,000-watt lamp with metal reflector and a 3-inch screen of water flowing through a pan with a clear glass bottom placed between the lamp and the plants. In the tests with *Rudbeckia* and sugar beets the improved lighting unit, affording better horizontal distribution of light intensity, was used. This unit consists primarily of a special type of cylindrical lamp that burns immersed in distilled water contained in a clear glass globe, the distilled water being cooled by means of tap water flowing through a copper coil immersed in its upper layer.

At 12 inches below the light source the average illumination was about 2,600 foot-candles with either type of lighting equipment. At 28 inches below the light source the illumination was about 1,200 foot-candles, and it decreased but little at greater distances below the light source. The air velocity in the cabinets was maintained at about 0.9 mile per hour. Computations indicate that at this rate of air flow the concentration of the carbon dioxide could not be appreciably modified by the action of the plants. When the temperature level was varied the relative humidity also was changed in such a way as to maintain a constant saturation deficit.

The wooden containers used for growing the soybeans were made watertight by coating them on the inside with asphalt and were fitted with metal covers containing 2-inch holes through which the plants grew. Split perforated corks were used to close the openings

and hold the plants in place. Aluminum containers asphalted on the inside were employed in growing *Rudbeckia* and sugar beets. Covers could not be used because of the growth characteristics of these plants.

Two potting-soil mixtures composed of a heavy loam, manure, and a small component of sand were used. The first mixture was employed in all the experiments with soybeans except the one run at 89° F. During the tests the moisture content of this mixture was maintained automatically at approximately 17.4 percent. The moisture content of the second soil mixture, employed in all the remaining experiments, was similarly maintained at 24 percent. In each instance the soil moisture was approximately 54 percent of saturation, and the weight of the moist soil in each container was approximately 80 pounds.

In the experiments with soybeans 5 plants of each variety, or a total of 15 individuals, were grown in each container. In the experiment with sugar beets at 65° F. 9 individuals were grown in each container, but in the remaining tests the number of plants was reduced to 4 in order to avoid crowding. In all the *Rudbeckia* tests 9 individuals were allowed to develop in each container.

EXPERIMENTAL DATA

SOYBEANS

In the six experiments with soybeans comprising the principal series to be reported in detail, the daily light periods ranged from 3 to 17 hours and the four temperature levels employed were 71°, 77°, 83°, and 89° F. In order to make a more detailed study of the response to day length at 77°, the interval between light periods was only 1 hour, and since only eight periods could be used in a single test, two separate experiments were required to cover the range from 3 to 17 hours. The first of these embraced light periods ranging from 3 to 10 hours, inclusive, and the second embraced periods ranging from 10 to 17 hours, inclusive. The experimental data presented in table 1 for the light periods ranging from 3 to 10 hours and a temperature of 77° represent the average results of duplicate tests. Three experiments covering the longer light periods were carried out, but in two of these the control of temperature and humidity was not entirely satisfactory because of abnormal outdoor conditions, so the data reported in the principal series cover only a single experiment. At temperatures other than 77° the interval between light periods was 2 hours, so that in each instance a single experiment sufficed to cover the entire range in light periods. At each temperature level the relative humidity employed was that corresponding to a saturation deficit of 0.424 mm of mercury. The duration of the experiments ranged from 40 to 60 days.

The essential conditions applying to the individual experiments are as follows:

Experiment 1: Duration, 60 days or 1,440 hours; temperature, 71° ±0.5° F.; relative humidity, 44 ±1 percent; light periods, 3 to 17 hours.

Experiment 2: Duration, 40 days or 960 hours; temperature, 77° ±0.5°; relative humidity, 54.2 ±1 percent; light periods, 3 to 10 hours.

Experiment 3: Duration, 44 days or 1,056 hours; temperature, 77° ±0.5°; relative humidity, 54.2 ±1 percent; light periods, 3 to 10 hours.

Experiment 4: Duration, 50 days or 1,200 hours; temperature, 77° ± 1°, except that for a period of 4 hours there was a mean departure of approximately +2°; relative humidity, 54.2±2 percent, except that for a total period of 50 hours there was a mean departure of about +4 percent; light periods, 10 to 17 hours.

Experiment 5: Duration, 59 days or 1,416 hours; temperature, 83° ±0.5°; relative humidity, 61±1 percent; light periods, 3 to 17 hours.

Experiment 6: Duration, 44 days or 1,056 hours; temperature, 89° ± 1°; relative humidity, 69±2 percent; light periods, 3 to 17 hours.

In these tests, as well as in those described later, the temperature and humidity levels and the range of departure from these levels

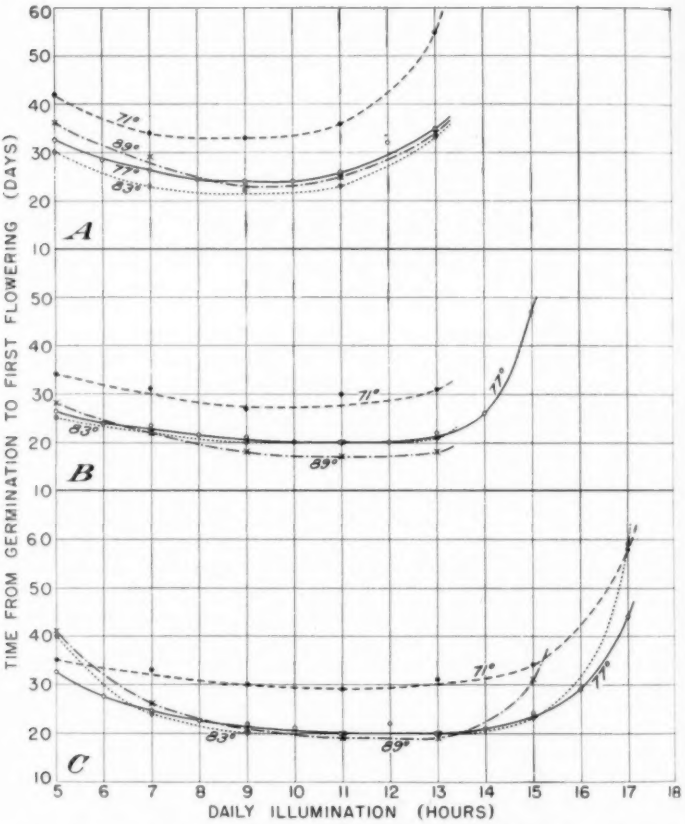

FIGURE 1.—Effect of daily light period on number of days elapsing from germination to appearance of first blossoms in Biloxi (*A*), Peking (*B*), and Mandarin (*C*) varieties of soybeans grown at various fixed temperatures.

pertain in all cases to the air as it entered the individual light cases. The possible disturbing effects of the lamps within the cases on the air temperature and humidity have been considered by Steinberg (*10*). Numerous observations within the cases have indicated that the temperature range incident to the going on and off of the lamps does not exceed ±0.5° F.

In the above-described experiments observations were made on the number of days elapsing from germination to appearance of first blossoms and from germination to the average time of flowering of all individuals in each treatment, on the height of the plants at time of flowering and at the end of the test, and on the number of nodes and length of internodes of the plants. An attempt was made to obtain also the fresh and dry weights of the plants at the end of the experiments, but the data were rejected as unreliable primarily because of heavy shedding of the foliage leaves. The data bearing on duration of the vegetative stage and on growth characteristics are summarized in table 1 and figure 1. Unfortunately height measurements at time of flowering were not obtained in the experiments conducted at a temperature of 77° F., but the final heights of the plants were measured.

TABLE 1.—*Effect of daily light period on average number of days elapsing from germination to first flowering, on height of plants at flowering and at harvest, on number of nodes produced, and on average length of internodes in the Biloxi, Peking, and Mandarin varieties of soybeans grown at various fixed temperatures*

Temperature (° F.)	Daily illumination	Average time from germination to first flowering			Height of plants at—						Nodes produced			Average length of internodes		
					Flowering			Harvest								
		Biloxi	Peking	Mandarin	Biloxi	Peking	Mandarin	Biloxi	Peking	Mandarin	Biloxi	Peking	Mandarin	Biloxi	Peking	Mandarin
	Hours	*Days*	*Days*	*Days*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Number*	*Number*	*Number*	*Cm*	*Cm*	*Cm*
71	5	51.8	34.6	41.0	42.9	28.5	28.1	46.2	32.9	31.4	6.4	5.6	4.6	7.2	5.9	6.9
	7	35.4	34.3	34.8	35.7	20.5	25.1	54.1	25.4	27.8	8.0	6.0	5.6	6.8	4.2	5.0
	9	33.6	30.0	31.4	41.0	24.0	26.2	81.8	37.6	34.1	10?8	7.0	6.6	7.6	5.4	5.2
	11	38.4	31.6	31.0	44.5	20.9	25.8	84.1	37.7	38.1	12.4	6.6	7.2	6.8	5.7	5.3
	13	55.6	32.6	33.4	88.3	26.3	26.7	105.4	46.0	45.3	16 6	7.5	7.6	6.4	6.1	6.0
	15	----	----	34.6	----	----	34.3	100.7	88.9	85.7	16.0	13.5	12.2	6.3	6.6	7.0
	17	----	----	59.3	----	----	98.8	106.8	106.2	107.0	16.8	15.0	15.8	6.4	7.1	6.8
77	4	43.0	33.5	----	----	----	----	48.2	28.1	34.8	4.7	4.0	4.0	10.3	7.0	8.7
	5	35.3	27.3	35.0	----	----	----	60.8	34.9	38.4	6.4	5.5	4.5	9.5	6.4	8.5
	6	29.4	25.0	28.8	----	----	----	65.9	37.5	41.4	7.2	6.0	4.9	9.1	6.3	8.4
	7	27.3	23.8	25.8	----	----	----	67.1	40.3	44.6	7.2	5.6	5.3	9.3	7.2	8.4
	8	26.0	22.8	23.5	----	----	----	77.2	42.8	45.6	8.6	6.0	5.9	9.0	7.1	7.7
	9	25.0	22.3	23 0	----	----	----	76.9	43.4	51.2	9.8	5.9	6.1	7.9	7.4	8.4
	10	26.7	21.2	22.5	----	----	----	94.0	52.8	64.2	10.4	6.0	7.0	9.0	8.8	9.2
	11	27.0	21.6	22.4	----	----	----	92.5	56.1	62.9	7.8	6.0	7.6	11.9	9.4	8.3
	12	32.7	20.8	24.4	----	----	----	117.3	62.8	84.6	10.0	6.2	8.3	11.7	10.1	10.2
	13	36.3	22.2	22.0	----	----	----	130.5	74.5	84.6	13.7	8.4	8.8	9.5	8.9	9.6
	14	----	27.0	22.0	----	----	----	80.0	96.3	80.4	8.3	9.8	8.5	9.7	9.8	9.5
	15	----	----	25.2	----	----	----	91.3	141.0	122.7	9.5	14.8	10.0	9.6	9.5	12.3
	16	----	----	32.0	----	----	----	113.0	128.5	141.6	12.5	17.0	14.2	9.0	7.6	10.0
	17	----	----	45.0	----	----	----	109.1	123.0	121.8	11.2	13.5	13.3	9.7	9.1	9.2
83	5	39.4	25.6	41.5	50.4	33.0	36.5	59.6	46.2	39.4	7.2	7.0	5.3	8.1	6.6	7.4
	7	27.0	23.2	25.3	51.0	31.6	43.8	100.2	49.7	54.6	11.6	7.8	6.0	8.7	6.4	9.1
	9	23.2	21.0	21.4	51.9	30.5	45.7	154.6	40.3	91.6	17.0	7.2	8.4	9.1	6.8	10.9
	11	25.2	21.0	21.4	61.6	31.8	40.3	126.3	46.5	72.8	17.6	6.8	8.4	7.2	6.8	8.7
	13	41.0	22.8	20.6	120.5	39.0	51.5	165.0	52.1	91.7	22.4	7.8	9.0	7.4	6.7	10.2
	15	----	----	25.6	----	----	69.1	143.0	136.4	179.0	23.0	15.0	15.2	6.2	9.1	11.8
	17	----	----	59.7	----	----	186.8	164.0	139.9	183.4	24.3	14.4	16.2	6.7	9.7	11.3
89	5	36.0	29.6	41.0	81.5	42.8	49.0	76.8	45.7	50.4	7.0	6.4	5.6	11.0	7.1	9.0
	7	33.6	24.2	28.0	95.4	40.4	66.3	125.3	58.3	71.8	11.0	7.8	5.6	11.4	7.5	12.8
	9	25.0	18.4	26.2	68.2	39.3	74.3	129.0	69.7	102.8	13.6	7.8	5.4	9.5	8.9	19.0
	11	27.2	17.6	20.2	88.5	48.1	84.1	145.7	82.5	93.6	13.2	8.4	7.0	11.1	9.8	13.4
	13	34.0	20.6	19.6	93.5	63.3	100.1	144.5	101.3	134.5	16.4	8.2	7.0	8.8	12.4	19.2
	15	----	----	34.3	----	----	127.5	158.0	170.3	186.3	15.6	13.6	11.2	10.0	12.5	16.6
	17	----	----	----	----	----	----	159.7	166.2	192.0	17.0	13.8	10.6	9.4	12.1	18.1

In view of the fact that the tray screen used in the soybean tests was less efficient with respect to horizontal distribution of illumination than the globular screen utilized in the experiments with *Rudbeckia*

and beet, a special experiment was made to compare the two types of screen in their effects on flowering in soybeans exposed to certain light periods. The essential conditions of the test were: Duration, 39 days or 936 hours; temperature, 77° ± 0.5° F.; relative humidity, 54.2 ± 1 percent; light periods, 5, 10, and 15 hours. The results are shown in table 2.

Ability to reproduce results obtained under given conditions perhaps constitutes one of the best means of testing the effectiveness of the control measures employed. The data on initiation of flowering obtained in experiments 2 and 3, the conditions of which already have been outlined, throw light on this phase of the investigation. The results are shown in table 3.

TABLE 2.—*Comparative effects of tray and globular types of water screen placed between light source and test plants on number of days elapsing from germination to appearance of first blossoms in Biloxi, Peking, and Mandarin soybeans exposed to various light periods and a temperature of 77° ± 0.5° F.*

Daily illumination (hours)	Biloxi		Peking		Mandarin	
	Tray screen	Globular screen	Tray screen	Globular screen	Tray screen	Globular screen
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
5	35	29	26	25	32	31
10	25	25	20	21	20	20
15					24	24

TABLE 3.—*Results of duplicate experiments, A and B, to determine effect of daily light period on number of days elapsing from germination to appearance of first blossoms in Biloxi, Peking, and Mandarin soybeans grown under controlled conditions at a temperature of 77° ± 0.5° F.*

Daily illumination (hours)	Biloxi		Peking		Mandarin	
	A	B	A	B	A	B
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
5	32	33	26	27	33	32
6	28	29	24	24	27	28
7	26	27	23	22	24	25
8	24	25	22	22	22	23
9	23	25	21	21	21	23
10	23	25	20	21	21	21

The illumination conditions obtained with the gas-filled tungsten lamp and water screens used in the present experiments differ decidedly from those of natural illumination, especially as to the composition of the light and the daily curve of illumination intensity. For this reason it is of considerable importance to determine whether the photoperiodic responses obtained with the artificial illumination are in line with those previously observed with sunlight. In table 4 comparison is made between the results obtained with a 10-hour daily illumination period in the present tests at 77° F. and those obtained at comparable temperatures with 10 hours of sunlight in outdoor summer plantings and with the full day of the winter months in greenhouse plantings. Similar agreement in effects of the artificial and natural illumination was obtained with other daily light periods.

TABLE 4.—*Comparison of natural and artificial illumination as affecting duration of the vegetative period of Biloxi, Peking, and Mandarin soybeans under short-day conditions*

Conditions of test	Mean temperature	Biloxi	Peking	Mandarin
	°F.	*Days*	*Days*	*Days*
Outdoor plantings at 1-day intervals through June and July, 10-hour day	75.1–80.4	23.6	19.2	19.4
Greenhouse plantings at 5-day intervals in December, January, and February, natural day of 9.5–12 hours	75–79	26.8	21.5	23.1
Controlled conditions, tungsten filament lamp, 10-hour day	77±0.5	24.0	20.0	21.0

SUGAR BEETS AND RUDBECKIA

The experiments with sugar beets were carried out at three temperature levels, and in each instance light periods of 6, 9, 12, 13, 14, 15, and 18 hours and continuous light were employed. In the first experiment, which was run at 65° F., a saturation deficit of 0.25 mm of mercury was maintained. It was found, however, that this saturation deficit would be excessive for the higher temperature used in the second test, since no artificial refrigeration was available. Consequently in the later tests, covering both the highest and lowest temperatures used, the saturation deficit was maintained at 0.183 mm. The essential conditions applying to the three experiments were as follows:

Experiment 1: Duration, 77 days or 1,848 hours; temperature, 65°±1° F.; relative humidity, 59±1 percent, except that for a total period of 52 hours there was an average departure of −0.4 percent.

Experiment 2: Duration, 100 days or 2,400 hours; temperature, 60°±1°, except that for an aggregate period of 100 hours there was an average departure of −1.8°; relative humidity, 65±1 percent.

Experiment 3: Duration, 56 days or 1,344 hours; temperature, 73°±0.5°; relative humidity, 76.5±1 percent. Although it was not possible to maintain accurate control of temperature and humidity beyond the period of 56 days, the cultures were continued under rough control for an additional period of 3 weeks to determine whether flowering would occur under conditions other than continuous light. In this interval the temperature range was 73° to 78° and that of the relative humidity 76.5 to 83 percent. However, there was no further flowering.

The significant data on flowering in the three experiments are given in table 5. For the duration of the tests there was no reproductive response under the light periods shorter than 18 hours.

TABLE 5.—*Effect of daily light period on number of days elapsing from germination to appearance of first blossoms in sugar beets grown at 60°, 65°, and 73° F.*

Daily illumination (hours)	60° F.	65° F.	73° F.
	Days	*Days*	*Days*
15	(1)	(1)	(1)
18	65	66	(1)
24	65	48	39

[1] Plants did not flower.

Only two experiments were conducted with *Rudbeckia*. The light periods used were the same as for the sugar beets. As in the tests with soybeans, the relative humidity was that corresponding to a saturation deficit of 0.424 mm of mercury.

Experiment 1: Duration, 60 days or 1,440 hours; temperature, 83° ± 0.5° F.; relative humidity, 61.2 ± 1 percent.

Experiment 2: Duration, 54 days or 1,296 hours; temperature, 77° ± 1°, except that there was an average departure of +1° for a total period of 8 hours; relative humidity, 54.2 ± 1 percent, except that for a total period of 84 hours there was an average departure of +4 percent.

The results obtained in the two experiments are shown in table 6. In the experiments at 77° F. stem elongation occurred with the 13-hour day, but the plants had not flowered when the experiments were terminated. There was no flowering with day lengths of 6, 9, or 12 hours at either temperature.

TABLE 6.—*Effect of daily light period on number of days elapsing from germination to appearance of first blossoms and from germination to average time of flowering of all individuals and average height attained by the stem of Rudbeckia bicolor grown at 77° and at 83° F.*

Daily illumination (hours)	Time elapsing from germination to first flowering at—		Time elapsing from germination to average date of flowering of all individuals at—		Average height of plants at time of—			
					Flowering at—		Harvest at—	
	77° F.	83° F.	77° F.	83° F.	77° F.	83° F.	77° F.	83° F.
	Days	*Days*	*Days*	*Days*	*Cm*	*Cm*	*Cm*	*Cm*
13		55		56.5		62	22	52
14	44	38	47	45.0	46	62	55	83
15	49	34	51	36.5	81	66	77	98
18	35	29	41	32.0	56	66	84	102
24	35	29	43	31.0	64	61	87	106

INTERPRETATION AND DISCUSSION OF RESULTS

For the most part the experimental material was grown under closely controlled conditions, and the data for soybeans given in table 3 show that with the equipment and methods employed it is possible to satisfactorily reproduce experimental results. That the growth and development of the experimental material obtained in the artificial climates employed closely approximate results under natural conditions is shown in figure 2. Although the improved lighting assembly with the globular water screen possesses several advantages over the older lighting system with the tray type of water screen, the latter also can be made to yield satisfactory results (table 2). It is a matter of special interest that with the artificial climates used in the present experiments the number of days elapsing from germination to appearance of first blossoms in the three varieties of soybean in response to favorable daily light periods agrees closely with results obtained with plantings grown with natural illumination under comparable temperature conditions (table 4) and previously reported in part (7). In this connection it may be stated that observed differences of 1 or 2 days in the time elapsing from germination to first flowering are not ordinarily significant, since it becomes largely a matter of individual judgment as to whether opening of a particular blossom is to be regarded as occurring on a given day or on the following day.

The number of days from germination to first flowering in each variety of soybean as affected by day length at each of the four temperature levels employed is shown graphically in figure 1. The corre-

sponding data for the average number of days required for all individuals in each test to reach the flowering stage are given in table 1. In view of the small number of plants used, the results as a whole are remarkably consistent. At each of the temperatures employed a selective or differentiating action of day length on the three different varieties with respect to the critical light period is clearly in evidence. With favorable temperature conditions the critical light period for the Mandarin lies in the region of a 17-hour day, that of the Peking around a 15-hour day, and that of the Biloxi between a 13- and 14-hour day. Additional observations with narrower differences in day length

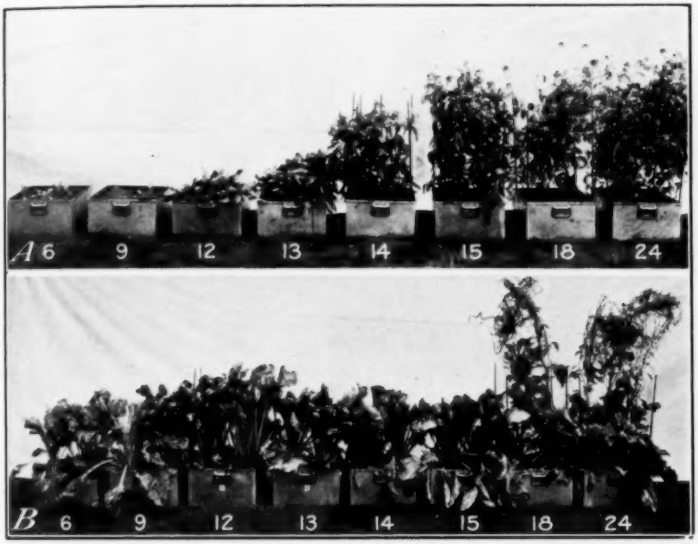

FIGURE 2.—*Rudbeckia bicolor* (*A*) and sugar beets (*B*) grown with various daily light periods ranging from 6 hours to continuous light, as indicated by numbers below the plant containers, and at fixed temperatures of 77° and 60° F., respectively. These cultures illustrate the type of growth and development obtained with the artificial climates employed.

are needed to fix more accurately these critical light periods for the particular conditions involved. As the temperature becomes increasingly unfavorable the critical light period tends to become somewhat shorter, as shown, for example, in the result with Mandarin at 89° F. and that with Biloxi at 71°. For each variety there is a rather wide range in day lengths that are shorter than the critical light period and are approximately optimum for flowering. For the Mandarin this range is about 9 to 14 hours, for the Peking 8 to 12 or 13 hours, and for the Biloxi 7 to 11 hours, although distinctly unfavorable temperatures tend to narrow somewhat these ranges. Perhaps the true optimum day-length ranges can be somewhat more narrowly defined as the zones lying between 11 and 13 hours for the Mandarin, 9 and 12 hours for the Peking, and 8 and 10 hours for the Biloxi.

It is of interest to consider the relative efficiency of the light energy in initiating reproductive activities under conditions of constant intensity and composition but with varying daily duration of light. Taking

for this purpose the product of the daily hours of light and the number of days elapsing from germination to appearance of first blossoms, it appears that, as shown graphically in figure 3, exclusive of the general region of the critical light period, the values for each variety and each temperature employed tend to form ascending linear series. The total number of light hours elapsing from germination to first flowering increases with increase in length of the daily light period, maximum efficiency of the light being attained with the shortest day length which will maintain the plant. Hence, graphs representing

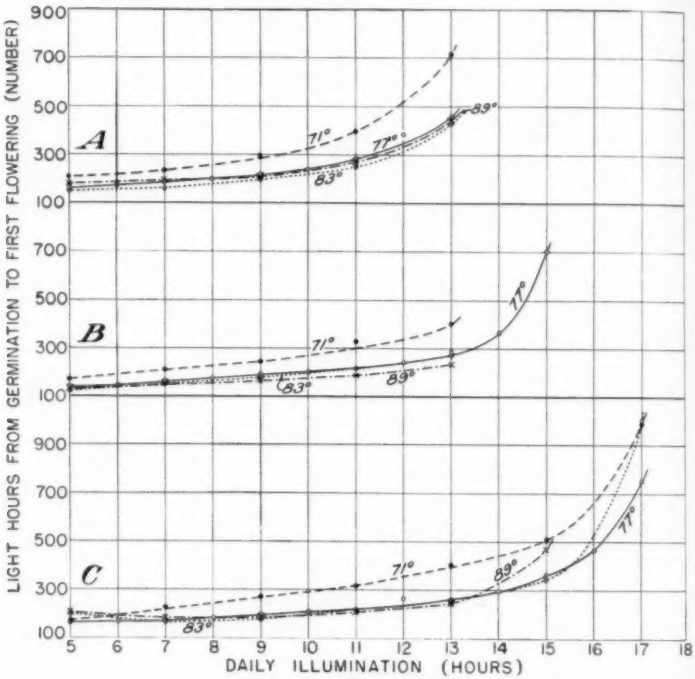

FIGURE 3.—Effect of daily light period on total number of light hours elapsing from germination to appearance of first blossoms in the Biloxi (*A*), Peking (*B*), and Mandarin (*C*) varieties of soybeans grown at various fixed temperatures.

this relationship differ in form from those of figure 1 except in the region of the critical light period, where they assume the same form. It seems probable that the increase in number of days elapsing from germination to first flowering with the shortest light periods employed as compared with the intermediate light periods (fig. 1) is due to conditions of impaired nutrition rather than to any weakening of the formative action of the light-darkness ratio. None of the soybean varieties was able to survive with a 3-hour daily period of light, while the Biloxi and Peking flowered with a 4-hour period and the Mandarin flowered with a 5-hour period.

As always has been the case when the soybeans were grown with natural illumination, the factor of heredity in relation to relative

number of days elapsing from germination to appearance of first blossoms in the different varieties under optimum conditions comes into play in the present data, although its effect is small. As shown by the values in figure 1 and the corresponding average values in table 1, the minimum number of days elapsing from germination to first flowering in the Biloxi exceeds the minimum in the Mandarin by about 3 days, and the minimum in the latter exceeds that in the Peking by 1 or 2 days.

The effect of temperature on the duration of the vegetative stage is clearly evident in the results obtained (table 1 and fig. 1). The lowest temperature employed (71° F.) was distinctly suboptimum, and flowering was delayed in all varieties and with all day lengths. The next higher temperature (77°) is much nearer the optimum for all varieties and all day lengths. The third temperature level (83°) gave practically the same results with Mandarin and Peking as were obtained at 77° for all day lengths except in the region of the critical light period, but 83° appears to be nearer the optimum than 77° for the Biloxi. The highest temperature employed (89°) was somewhat excessive on the whole, especially for the Biloxi, although at optimum day lengths this temperature slightly accelerated flowering in the Peking and Mandarin as compared with lower temperatures.

The interrelation of temperature and day length as affecting soybeans has been studied by Eaton (*2*) and by Gilbert (*8*). By subjecting Peking soybeans that were growing in a 13-hour day to a night temperature of about 50° F., Eaton obtained a considerable delay in flowering in comparison with similar plants exposed to night temperatures of 65° and 90°, respectively. Gilbert made observations on Mandarin, Peking, and Biloxi soybeans grown under short-day conditions in the greenhouse, one lot of each being exposed to a mean temperature of 65° to 70° and a relative humidity of 85 percent, while corresponding material received a mean temperature of 75° to 80° and a relative humidity of 50 percent. Under the latter conditions all varieties showed flower buds in about 30 days, while at the end of this period there was no indication of flowering at the lower temperature and higher humidity.

It is apparent that the soybean is decidedly a warmth-loving type. In the data herein presented there is no evidence of a definite selective or differentiating effect of temperature on the three varieties as to time elapsing from germination to appearance of first blossoms. Apparently the effects of temperature could not explain the fact that when planted in early summer in the latitude of Washington, D. C., the Mandarin, Peking, and Biloxi invariably behave as early, medium, and late-maturing varieties, respectively. The falling temperature in late summer or fall would tend to retard rather than hasten flowering in the late variety, while the relatively high temperature of midsummer would favor early flowering in this variety. In line with previous findings (*7*) it appears that length of day rather than temperature is primarily responsible for the observed differences in the three varieties with respect to time of flowering and fruiting when grown in moderately high latitudes. However, it seems clear that a relatively high mean temperature is required for success in all of these varieties.

The data in table 1 afford a basis for computing temperature coefficients for flowering of the Biloxi, Peking, and Mandarin soybeans

through a range in temperature of 18° F. at various day lengths. These coefficients are shown in table 7. It is evident that all are below the values (2–3) ordinarily associated with chemical reactions, and progressively increase in magnitude with increase in day length. If the size of the temperature coefficient be indicative of the type of reaction, as is usually assumed, it would seem that the reactions that are associated with flowering in the plant differ from those applying to vegetative growth. With increasing day length the purely chemical reaction appears to play an increasingly greater part in determining the time of the flowering process, as indicated by the increasing values of the temperature coefficients. It is felt that the divergencies in 2 of the 16 values do not vitiate these conclusions inasmuch as the remaining data are relatively uniform even though based on only two experiments, and, moreover, the variations occur near the region of greatest error, namely, the critical day length for flowering. Again the uniformity in values for the three varieties at each of the day lengths tested indicates that flowering is influenced by temperature to approximately the same degree in all these varieties, and that their differentiation in nature into late, medium, and early varieties, respectively, cannot be dependent on temperature.

TABLE 7.—*Temperature coefficients of flowering in soybeans at various day lengths for a range of 18° F.*

Daily illumination (hours)	Biloxi			Peking			Mandarin		
	Time elapsing from germination to flowering at—		Coefficient	Time elapsing from germination to flowering at—		Coefficient	Time elapsing from germination to flowering at—		Coefficient
	71° F.	89° F.		71° F.	89° F.		71° F.	89° F.	
	Days	*Days*		*Days*	*Days*		*Days*	*Days*	
5	42	36	1.17	34	28	1.21	35	41	[1] 1.17
7	34	29	1.17	31	22	1.41	33	26	1.27
9	33	23	1.43	27	18	1.50	30	21	1.43
11	36	25	1.44	30	17	1.76	29	19	1.53
13	55	34	1.62	31	18	1.72	31	19	1.63
15							34	31	1.10

[1] Equals 0.85, and indicates a delay rather than a hastening in the time of flowering.

Because of the small number of individuals grown in each test, conclusions to be drawn from the data on growth relations necessarily must be somewhat tentative. As a result of crowding and other factors, individuals that obtained a poor start often tended to lag in growth; so the average values do not present smooth series such as were obtained in the data on flowering. Whether the plants under the various treatments attained the flowering stage, and also the time required to reach this stage, would be expected, of course, to modify the rate and especially the ultimate amount of growth. At 89° F. the attained height of the Mandarin at time of flowering increased progressively with increase in the daily number of hours of light (table 1), and apparently attenuation was associated with this response. In no other instance was there definite increase in attained height of the plants at flowering time in response to increase in the daily light period except in the general region of the critical

light period. Within the latter range of day lengths the height increased rapidly with increase in the light period. For reasons which are not clear these results differ somewhat from those obtained by various investigators working with natural illumination. Under the latter conditions increase in the light period through the lower ranges usually has resulted in increased height of the plants at time of flowering. However, the average daily rate of growth during the preflowering stage showed a clearly defined tendency to increase with increase in the photoperiod in all of the varieties at 83° and 89°. At 71° temperature apparently was the principal limiting factor, and only the Biloxi showed appreciable increase in growth rate in response to increase in day length. With respect to effects of temperature, with only a single exception, each increment increased both the attained height at flowering and the average daily gain in height for each day length employed.

The final height at time of harvest on the whole tended to increase with increase in the duration of the daily light period. In the Mandarin and Peking the rate of increase with increase in the light period was much more marked as the critical light period was approached, whereas in the Biloxi the height increased more or less uniformly with increase in duration of the light period. Interpretation of the data bearing on the relation of temperature to final height of the plants is complicated somewhat by the fact that the tests conducted at different temperatures were not continued for the same number of days. The average duration of all tests was approximately 50 days. When the data on final height in table 1 are adjusted on this basis, it appears that with all varieties and all day lengths employed increase of temperature from 71° to 77° F. materially increased the height of the plants. Further increase in temperature from 77° to 83° produced comparatively little effect. The final temperature increase from 83° to 89°, however, produced a further marked increase in plant height, this effect increasing somewhat with increase in day length. Even the unadjusted data of table 1 show on the whole a decided increase in height of the plants with increase in temperature.

The effects of varying the daily period of light on the number of nodes produced by the individual plant were similar to the effects on final height of the plants. In the Biloxi the number of nodes tended to increase more or less uniformly with increase in day length, whereas in the Mandarin and Peking the relative increase in number of nodes produced with increasing day length was much more marked in the region of the critical light period. The effects of temperature on the number of nodes produced and the average length of internodes become more clearly defined when the observed data are adjusted to a basis of 50 days as the duration of each test, as was done in considering temperature effects on the final heights of the plants. On the whole, the number of nodes, especially in the Biloxi, tended to increase with the increase in temperature, and in the case of the other two varieties with the increase from 71° to 77° F. At the higher temperatures employed, especially at 89°, increase in the photoperiod produced a pronounced increase in the average length of internodes in the Peking and Mandarin, but in other cases showed little or no consistent effect. Increase in temperature from 71° to 77° produced a definite increase in length of internodes in each variety and at all

day lengths, and there was a further increase in internode length, mainly in the Mandarin, at 89°, the highest temperature employed. On the whole, length of internode was least affected by temperature in the Biloxi and most affected in the Mandarin.

The data obtained with sugar beets are too limited to afford a comprehensive picture of the interrelationship of temperature and duration of illumination as affecting reproductive activity, but they throw considerable light on the subject. The beet is recognized as being a pronounced long-day type, and it has been found by several investigators also that in this plant stem elongation, flowering, and seed formation are favored by relatively low temperature. In the present tests flowering occurred in 65 days with an 18-hour day and with continuous illumination at a temperature of 60° F., but with increase of temperature the 18-hour light period progressively decreased in effectiveness. With continuous light, however, the time elapsing from germination to appearance of first blossoms decreased with increase in temperature up to 73°. It does not follow, of course, that maximum amount of flowering and fruiting would occur at the higher temperature. Under the conditions and for the duration of the tests there was no stem elongation or flowering with light periods shorter than 18 hours. In all cases there was considerable enlargement of the root.

In earlier experiments in the greenhouse, conducted by Garner in collaboration with Allard, the results of which have not been published in detail though briefly referred to in a nontechnical paper (*3*), several varieties of table beet as well as sugar beet developed seedstalks and flowered freely without material thickening of the root when exposed to a 16- to 17-hour day and a mean temperature of about 55° F. The plants received the full day of winter supplemented with high-intensity artificial light till midnight, and the daily range in temperature was about 10°. The time required for flowering was 130 to 135 days from germination. With similar illumination and a mean temperature of approximately 72° no stem elongation or flowering occurred, but there was decided enlargement of the root and the leaves were quite long. Under the short-day conditions there was no stem elongation at either temperature during the period of the tests, while there was more pronounced thickening of the root at the higher than at the lower temperature. In a prior experiment with the table beet (*6*) large roots taken from winter storage and grown under outdoor conditions of temperature and full day length in late spring soon flowered. Similar plants exposed to a 10-hour day produced short seedstalks, but the apical buds subsequently developed into leaf rosettes and there was no flowering.

With a day length of 14.5 to 15 hours and a mean temperature of 65° F. or slightly lower, Skuderna [3] obtained flowering within 75 days in sugar beet seedlings growing in the greenhouse. Supplementing daylight with high-intensity electric light for the entire night and avoiding the high temperatures of midsummer, Munerati (*9*) was able to grow five generations of beets in 1 year, obtaining mature seed in 52 to 66 days after planting. With a temperature of 50° to 66°, Chroboczek (*1*) obtained elongation of seedstalks but no flowering in table beets exposed to an 8-hour day, and sugar beets failed to produce

[3] SKUDERNA, A. W. PHOTOPERIODISM. Amer. Beet Sugar Co., Research Dept. 1926. [Unpublished.]

seedstalks under the short-day conditions. With continuous illumination flowering occurred, even at a temperature of 70° to 80°.

It seems clear that the beet, which is a long-day type, differs also from the soybean, a short-day type, in that the temperature range favorable to reproductive activity in the former is considerably lower than that favoring reproduction in the latter. On the other hand, it appears that within the favorable range of temperature the time elapsing from germination to appearance of first blossoms in the beet tends to decrease with increase of temperature in much the same way as in the soybean. In either case change in temperature may modify to some extent the response to length of day, and vice versa, although it does not necessarily follow that the mode of action of temperature and of day length is the same. Apparently the beet does not require a winter rest period and commonly behaves as a biennial only because in most situations it is grown under combined conditions of light and temperature that are more or less unfavorable for reproductive activity.

The data in table 6 indicate that the critical light period for *Rudbeckia* is 13 hours or somewhat shorter, at least for the temperatures employed. Beyond this point increase in the day length up to 18 hours decreases the time elapsing from germination to appearance of first blossoms, but the 18-hour day is as effective as continuous light. The final height of the plants increases with increase in day length up to 18 hours. Increase in temperature from 77° to 83° F. decreases the time elapsing from germination to first flowering and increases the final height of the plants. *Rudbeckia*, a long-day type, resembles the beet in its response to length of day, while it rather closely resembles soybeans in its temperature requirements. As in the case of both the beet and the soybean, within the range of temperature favorable to reproductive activity the time elapsing from germination to first flowering decreases with increase of temperature.

From the standpoint of the interrelationship of length of day and temperature as environmental factors, soybeans, sugar beets, and *Rudbeckia* may be regarded as representing three somewhat contrasted types with respect to reproductive response. For the beet, a combination of long day (or continuous light) and cool temperature affords optimum conditions; for *Rudbeckia bicolor*, a combination of long day and warm temperature; and for late-maturing (Biloxi) soybeans, a combination of short day and warm temperature. The early-maturing (Mandarin) soybean differs essentially from the late-maturing variety only in that it has a considerably longer critical light period, and in this respect the medium variety (Peking) occupies an intermediate position. The fourth possible type of plant, in which reproductive activity is favored by a short day combined with cool temperature, is not represented in the present experiments. The work of Gilbert (*8*) indicates that *Cosmos* is a representative of this type.

During the open growing season of temperate regions the mean temperature tends to decrease with increase in latitude, although locally such factors as altitude may modify this tendency. There is definite increase in length of day, of course, with increase in latitude. Accordingly, in most situations the beet would be more likely to behave as an annual at very high latitudes. For *Rudbeckia bicolor* the favorable effect on reproductive activity of the long days of very high latitudes might be offset by the lower mean temperature. In high

latitudes the lower temperature would be unfavorable alike to the early and late varieties of soybeans, but the latter, because of their shorter critical light period, would be at a distinct disadvantage as compared with the former. Plants of the type to which *Cosmos* belongs probably are better adapted to relatively high latitudes than is the group represented by the late-maturing variety of soybeans, for although the two groups have similar day-length requirements the falling temperature of late summer or early fall is more favorable for the former.

SUMMARY

Fairly extensive observations were made on response to length of day of early-, medium-, and late-maturing varieties of soybeans grown at mean temperatures of 71°, 77°, 83°, and 89° F. under controlled conditions. Limited observations also were made on sugar beets grown at mean temperatures of 60°, 65°, and 73°, and on *Rudbeckia bicolor* Nutt. grown at mean temperatures of 77° and 83°. The plants were grown with high-intensity illumination from gas-filled tungsten-filament lamps in specially constructed chambers, and various day lengths were used.

With the artificial climates employed the reproductive response of soybeans to length of day agreed closely with results previously obtained with natural illumination under comparable conditions. With the artificial climates it was found possible to satisfactorily duplicate results.

At each of the temperatures employed there was definite contrast in the response of the three varieties of soybeans with respect to the critical day length for flowering, that is, the maximum length of day at which the plants tended to become reproductive. With favorable temperature conditions the critical light period for the early variety (Mandarin) was found to be in the region of a 17-hour day; that of the medium variety (Peking), around a 15-hour day; and that of the late variety (Biloxi), between a 13-hour and a 14-hour day. The optimum day length for flowering was not sharply defined, but that of the Mandarin seemed to range from approximately 11 to 13 hours, that of the Peking from 9 to 12 hours, and that of the Biloxi from 8 to 10 hours.

The soybean was found to have a relatively high temperature requirement, and there appeared to be only slight differences in the requirements of the three varieties used in the tests. The lowest mean temperature employed (71° F.) delayed flowering in each of the varieties at all day lengths. Temperature differences altered the critical light periods to a limited degree, but there were no marked contrasts in these effects on the three varieties. Response to length of day appears to be primarily responsible for the fact that the Mandarin, Peking, and Biloxi normally behave as early, medium, and late varieties, respectively.

The total number of light hours elapsing from germination to appearance of first blossoms increased with increase in length of the daily light period, maximum efficiency of the light being attained with the shortest day length capable of maintaining the plant. The efficiency of the light energy increased with increase of temperature.

Because of the small number of individuals grown in each test and their uneven growth rate, only tentative conclusions may be drawn from the data obtained on growth relations in the soybeans. At the

higher temperatures the average daily growth rate during the pre-flowering stage increased with increase in day length, but at 71° F. temperature appeared to be the chief limiting factor. The final height of the plants tended to increase with increase in the daily light period. The height at time of flowering as well as the final height tended to increase with increase in temperature. Increase in length of day increased the number of nodes produced, but did not greatly affect the length of the internodes except that in the Mandarin and Peking there was a marked increase at the higher temperatures. Increase in temperature also tended to increase both the number of nodes and their length, the latter effect being most obvious with the increase from 71° to 77°.

The sugar beet has been found to be a pronounced long-day type, and it also differs from soybeans in that the range of temperature favorable to reproductive activity is relatively low. The strain of sugar beet used in the tests flowered in 39 days when exposed to continuous light and a mean temperature of 73° F. As the daily light period decreased, lower temperature became increasingly favorable to flowering. Apparently the beet commonly behaves as a biennial rather than as an annual only because in most situations it is grown under combinations of day length and temperature that are more or less unfavorable to reproductive activity.

Rudbeckia bicolor resembles the beet in being a long-day type, while its temperature requirements are very similar to those of soybeans. The final height of the plants was found to increase with increase in day length up to 18 hours and with increase of temperature from 77° to 83° F.

From the standpoint of the interrelationship of length of day and temperature as environmental factors, soybeans, *Rudbeckia bicolor*, and beet represent three somewhat contrasted types with respect to reproductive activity. In late-maturing soybeans flowering and fruiting are favored by a combination of short day and warm temperature, in *Rudbeckia* by a combination of long day and warm temperature, and in the beet by a combination of long day and cool temperature. However, within the temperature range favorable to reproductive activity, whether this be warm or cool, in each instance increase in temperature hastens reproductive processes. The critical light period for flowering may be altered to a limited degree by temperature and, conversely, the favorable temperature range for flowering may be shifted by the action of day length.

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SCRIBNER VOLUME TABLES FOR CUT-OVER STANDS OF PONDEROSA PINE IN ARIZONA [1]

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INTRODUCTION

In connection with recent growth studies of cut-over stands of ponderosa pine (*Pinus ponderosa* Laws.) in Arizona, the Southwestern Forest and Range Experiment Station found it expedient first to construct new volume tables to fit the cut-over stands. The necessary measurements were made on an existing logging operation on felled timber similar in form to the residual stand. This was in line with Meyer's [2] findings in his analysis of data on ponderosa pine forests in the Pacific Northwest, that volume tables of this species may apply equally well to virgin stands or cut-over stands in that type, provided an adjustment is made in computing stand volumes where the cutting has greatly disturbed the balance of form classes.

On the basis of these data [3] it is the purpose of this paper to present (1) Scribner board-foot volume tables obtained from an equation (having only a small residual error [4]) adapted to the computing of volume increment in cut-over stands of ponderosa pine, and (2) a comparison of the "blackjack", "intermediate", and "yellow pine" forms of the species as to volume. These three terms, in common use locally, are employed to designate the three distinctive growth forms of ponderosa pine, namely, the very tapering immature trees with their dark, deeply furrowed, and narrow-plated bark; the transitional form with reduced taper and lighter bark; and the mature "yellow" pine, full-boled, with wide-plated, shallow-furrowed, yellowish-brown bark.

CHARACTER OF DATA

The data used in this investigation were collected in 1932 from trees felled during lumbering operations of the Cady Lumber Corporation on five sections on the Sitgreaves National Forest, Ariz. The stand was pure ponderosa pine, considered typical of the volcanic formation of the Southwest known as the Malpais soil type. The height of mature dominant trees averaged 95 feet. The area is gently rolling with low flat-topped hills and wide shallow canyons. Its aspect is in general southwesterly, and the altitude is about 7,500 feet.

As indicated above, since the volume tables were to be applied to cut-over stands, measurements were made only on trees (selected at random) of a type approximating as nearly as possible that of the trees reserved in Forest Service cutting, i. e., normal, live-topped trees free from external defects. Care was taken to reject trees with

[1] Received for publication Mar. 2, 1936; issued July 1936.

[2] MEYER, W. H. EFFECT OF RELEASE UPON THE FORM AND VOLUME OF WESTERN YELLOW PINE. Jour. Forestry 29: 1127–1133, illus. 1931.

[3] Acknowledgment is due the staff of the Southwestern Forest and Range Experiment Station and the personnel of the Sitgreaves National Forest for help in collecting the field data.

[4] "Residual error" as used here refers to variations in volume that are not dependent upon variations in diameter and height.

defects (such as bad fire scars, dead or bayonet tops, forked tops, or severe lightning injury) that would result in abnormality of diameter, height, or bark thickness.

With mean ground level as the base, diameter and bark measurements were taken on each tree with adjusted calipers to the nearest 0.1 inch at heights of 1, 3, 4.5, and 10 feet, and at intervals of 8 feet above that, with, usually, a shorter interval at the tip. The value recorded in each case was the mean of two measurements taken at right angles. The diameters at breast height [5] were remeasured with a diameter tape as a check on the caliper measurements. Bark thickness was measured to the nearest 0.1 inch with a Swedish bark-measuring instrument; the figure recorded for bark thickness was the sum of two measurements taken at points diametrically opposite. Total height was measured to the nearest 0.1 foot with a steel tape.

The three-man crew collecting the field data were experienced both in Forest Service timber-marking practice and in ponderosa pine timber-sale work in Arizona, New Mexico, and Idaho. In classifying trees as blackjack, intermediate, or yellow pine, each tree was analyzed on the basis of character and color of bark, shape and condition of crown, and character of tip. In case of disagreement among the three men, the classification agreed on by two was accepted. Since age was found to be a fair criterion of classification, judgment on this point was checked by a count of annual rings on the stump. Diameter and height growth of ponderosa pine is normally slow; the range and mean age of the three groups was found to be: 40 to 165 years, with a mean of 109, for blackjacks; 110 to 250 years, with a mean of 176, for intermediates; and 180 to 410 years, with a mean of 270, for yellow pines.

ANALYSIS OF DATA [6]

Each tree for which data were plotted was scaled by the Scribner log rule in 16-foot saw logs with trimming allowance of 0.3 foot, a stump height of 1 foot, and a top diameter of 8 inches inside bark. Top sections less than one full saw log were scaled as fractions of a 16-foot 8-inch saw log. A multiple correlation [7] of logarithm of (volume—6.6) with logarithm of (diameter breast high inside bark—8 inches) and logarithm of (height—4.5 feet) was made for 119 blackjacks by methods described by Schumacher and Hall.[8] The calculated statistics are given in table 1.

TABLE 1.—*Means and standard deviations of the logarithms of (diameter breast high inside bark, in inches—8), (total height, in feet—4.5), and (volume in board feet—6.6); and gross correlation between logarithms of the variables, for 119 blackjacks*

Variable (X)	Mean (M)	Standard deviation (σ)	Correlation coefficient (r)
	Logarithm	*Logarithm*	
X_1=Logarithm of (volume in board feet—6.6)	M_1=1.9911	σ_1=0.5160	r_{12}=+0.9557
X_2=Logarithm of (diameter breast high inside bark in inches—8)	M_2= .6215	σ_2= .3787	r_{13}=+ .7289
X_3=Logarithm of (total height in feet—4.5)	M_3=1.7589	σ_3= .0990	r_{23}=+ .6199

[5] 4.5 feet above the ground; abbreviated d. b. h.

[6] Acknowledgement is due F. X. Schumacher and R. A. Chapman of the Forest Service for assistance and valuable suggestions in the statistical analysis.

[7] In which the origin of coordinates is placed at 8 inches d. b. h., 4.5 feet in total height, and 6.6 board feet in volume.

[8] SCHUMACHER, F. X., and HALL, F. DOS S. LOGARITHMIC EXPRESSION OF TIMBER-TREE VOLUME. Jour. Agr. Research 47: 719–734, illus. 1933.

The logarithmic regression equation for these statistics, computed according to the method given by Yule,[9] is

$$\log(\text{volume}-6.6)=b_{12.3}\log(\text{diameter breast high inside bark}-8)+b_{13.2}\log(\text{height}-4.5)+\log(\text{of the constant term}),$$

or

$$X_1=1.1151X_2+1.1555X_3-0.7343,$$

and the multiple correlation coefficient $R_{1.23}=+0.9715$. The coefficient of multiple correlation measures the combined importance

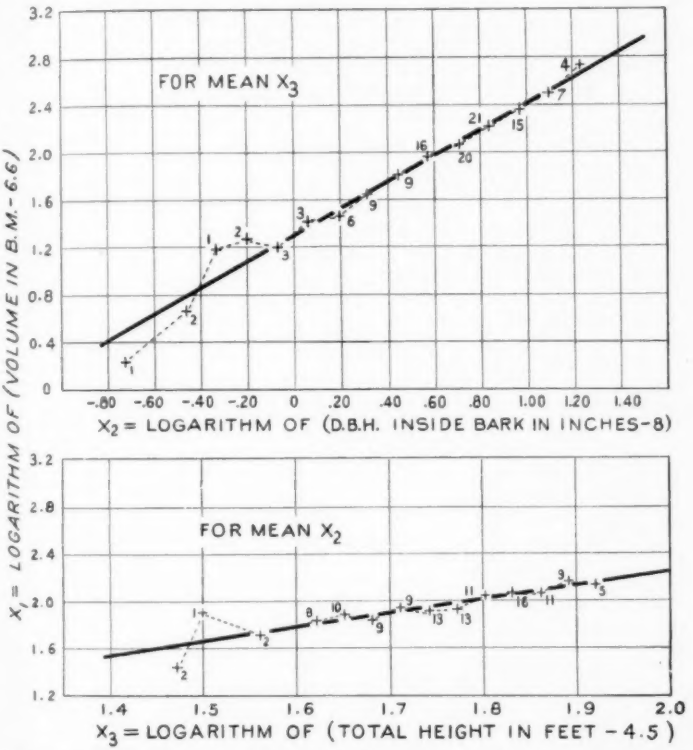
FOR MEAN X_3
X_1 = LOGARITHM OF (VOLUME IN B.M. - 6.6)
X_2 = LOGARITHM OF (D.B.H. INSIDE BARK IN INCHES-8)
FOR MEAN X_2
X_3 = LOGARITHM OF (TOTAL HEIGHT IN FEET - 4.5)

FIGURE 1.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 119 blackjack trees.

of X_2 and X_3 as a means of explaining the differences in X_1, 0 and 1 equaling no correlation and perfect correlation, respectively.

The test of the linearity of the relationship of the logarithm of (volume −6.6) to the logarithms of (diameter breast high inside bark −8) and (height −4.5) was made by comparing the actual X_1 value of each tree with the estimated X_1 value. The estimated X_1 values were read from an alinement chart constructed from the volume equation. The results are presented in figure 1. The fit of the data to the calculated equation is very good.

[9] YULE, G. U. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 8, rev., 422 pp., illus. London. 1927. (See pp. 229–252.)

The same type of equation was fitted to the data of the intermediate and yellow pine groups and to the combined group of blackjacks and intermediates. The graphic tests for linearity are given in figures 2, 3, and 4, in which, as also in figure 1, the dependent variable is the logarithm of board-foot volume by the Scribner log rule to a constant top diameter of 8 inches, less 6.6 board feet. In every case the fit

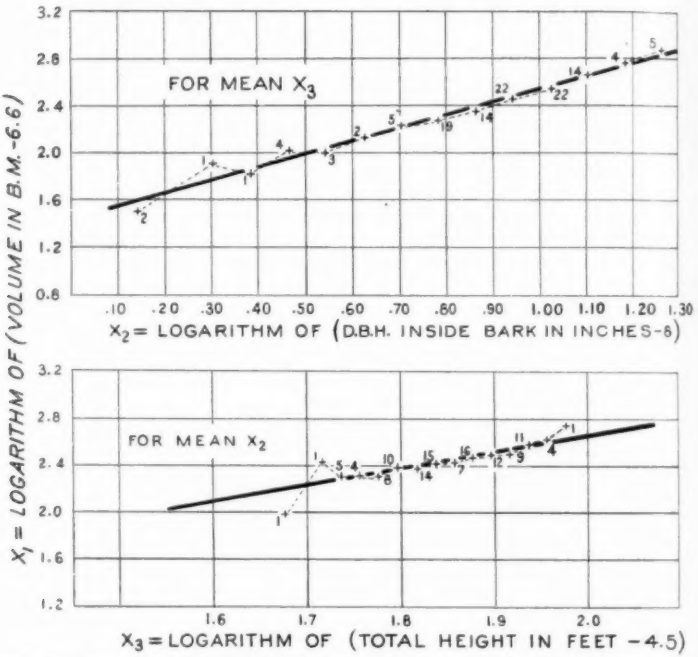
FOR MEAN X_3
FOR MEAN X_2
X_1 = LOGARITHM OF (VOLUME IN B.M.-6.6)
X_2 = LOGARITHM OF (D.B.H. INSIDE BARK IN INCHES-6)
X_3 = LOGARITHM OF (TOTAL HEIGHT IN FEET -4.5)
3.2
2.8
2.4
2.0
1.6
1.2
0.8
.10 .20 .30 .40 .50 .60 .70 .80 .90 1.00 1.10 1.20 1.30
1.6 1.7 1.8 1.9 2.0

FIGURE 2.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 118 intermediate trees.

of the data to a straight line is excellent and confirms the hypothesis presented by Schumacher and Hall.[10] [11] The logarithmic regression coefficients, the constant term, and other statistics for the data of each of these groups are given in table 2.

[10] SCHUMACHER, F. X., and HALL, F. DOS S. See footnote 8.

[11] "Timber-tree volume equations of the power or logarithmic type in terms of diameter and height are deduced from known volume equations of geometrical solids on the theory that the volume of the tree stem, unlike that of the geometrical solids, may vary not directly as height and as the square of diameter, but as other powers of these dimensions in both cubic feet and board feet. The theory is tested by transforming the power equation into a linear one in logarithms and calculating the logarithmic regression equations for available tree data."

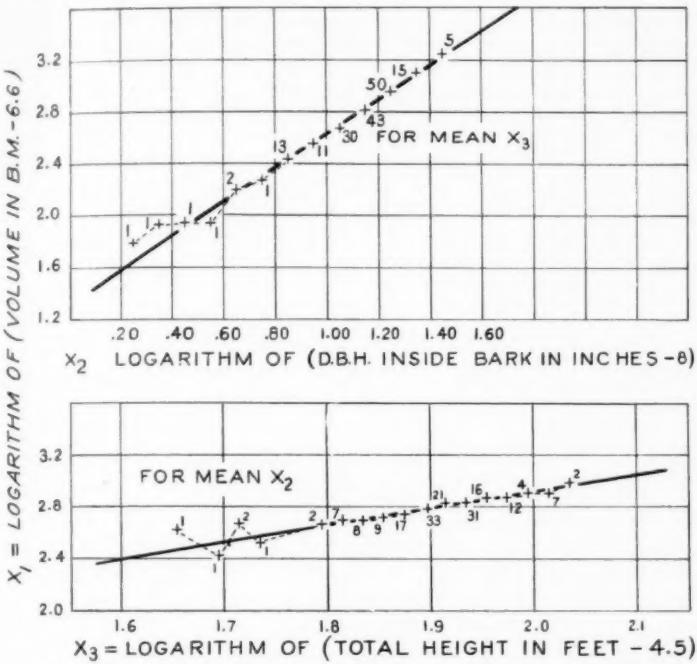

FIGURE 3.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 174 yellow pine trees.

TABLE 2.—*Logarithmic regression coefficients, constant terms, and other statistics for blackjacks, intermediates, and yellow pines, and blackjacks and intermediates combined, for each of which groups the logarithmic equations of Scribner board-foot volume were calculated*

Class	Partial regression coefficients		Constant term	Means		
	$b_{12.3}$	$b_{13.2}$		M_1	M_2	M_3
				Log	*Log*	*Log*
Blackjack	1.1151	1.1555	−0.7343	1.9911	0.6215	1.7589
Intermediate	1.1068	1.3627	−1.0793	2.4363	.8996	1.8492
Yellow pine	1.3183	1.2943	−1.1479	2.8002	1.1220	1.9076
Combined blackjack and intermediate	1.1201	1.3329	−1.0596	2.2081	.7694	1.8050

Class	Standard deviations			Trees	Standard error of estimate	Gross correlation coefficient	Multiple correlation coefficient	Coefficient of determination
	σ_1	σ_2	σ_3		$\sigma_{1.23}$	r_{23}	$R_{1.23}$	$R^2_{1.23}$
	Log	*Log*	*Log*	*Number*	*Log*			
Blackjack	0.5160	0.3787	0.0990	119	0.1224	+0.6199	0.9715	0.9437
Intermediate	.3169	.2154	.0623	118	.0863	+.6544	.9622	.9259
Yellow pine	.3203	.1907	.0610	174	.0700	+.7117	.9758	.9522
Combined blackjack and intermediate	.4837	.3371	.0938	237	.1032	+.6895	.9770	.9545

The coefficient of determination, $R^2_{1.23}$, shows the percentage of variance in log (volume −6.6) that is associated with variance in log (diameter breast high inside bark −8) and log (height −4.5). The coefficient of determination for yellow pine is shown by table 2 to be 95.22 percent. Thus only 4.78 percent of the variance in log (volume −6.6) is due to factors other than log (diameter breast high inside bark −8) and log (height −4.5). Accordingly these factors were

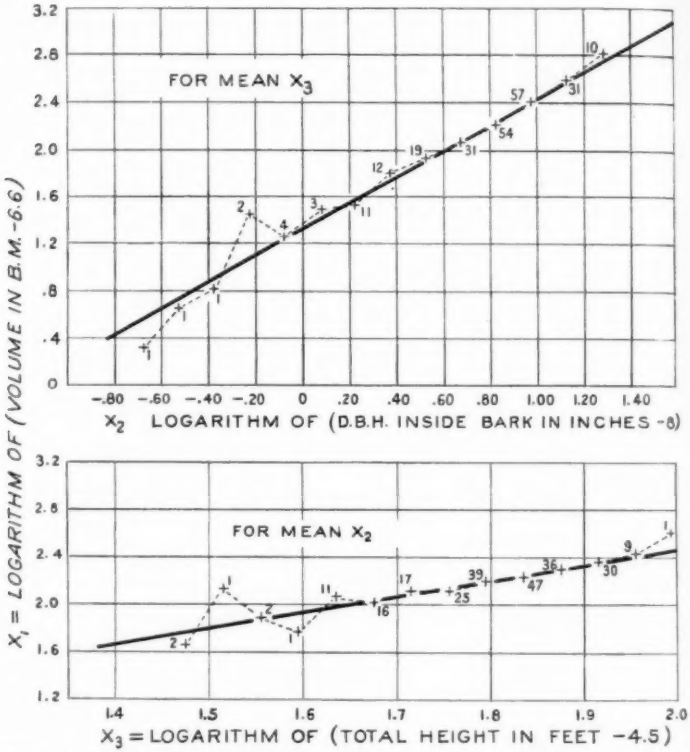
FOR MEAN X_3
X_1 = LOGARITHM OF (VOLUME IN B.M.-6.6)
X_2 LOGARITHM OF (D.B.H. INSIDE BARK IN INCHES -8)
FOR MEAN X_2
X_3 = LOGARITHM OF (TOTAL HEIGHT IN FEET -4.5)

FIGURE 4.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 237 blackjack and intermediate trees.

not considered in these computations. It should be noted, however, that the standard error of estimate, $\sigma_{1.23}$, is a measure of the accuracy with which the estimated log (volume −6.6) agrees with the actual log (volume −6.6) of the trees used in the study. From table 2 we find that the logarithmic volume equation for yellow pine is

$$X_1 = 1.3183\,X_2 + 1.2943\,X_3 - 1.1479,$$

and that the standard error of estimate is ± 0.0700.

COMPARISON OF VOLUME EQUATIONS

Comparison of the volume equations of blackjacks, intermediates, and yellow pines resolves itself into a test of the significance of the difference in the log (volume −6.6) of individual trees of a given diam-

eter breast high and height, as estimated by the volume equation calculated for each group. This test was accomplished through the use of the standard error of the function, i. e., of the regression equation, as given by Miner[12] and as applied to volume-table tests by Schumacher and Hall.[13]

With the logarithmic equations as the functions and twice the standard error of the difference between values as calculated by two

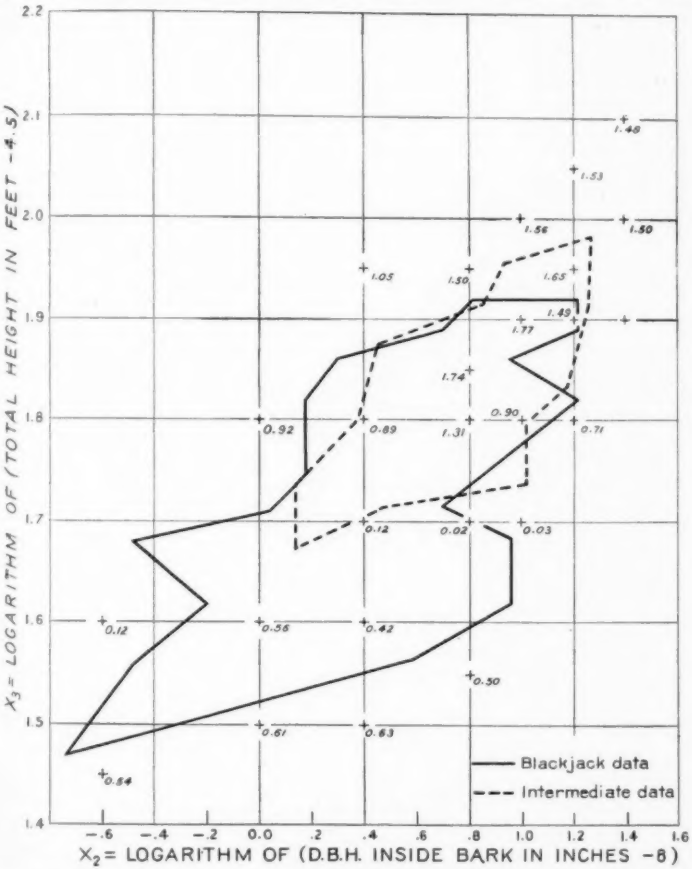

FIGURE 5.—Graphical representation of the test for determining the significance of the difference between the volume equation of the blackjack group and of the intermediate group. The volumes computed by the equations for these two groups are not significantly different.

functions for trees of the same size as the lower limit of significance,[14] tests were made between blackjacks and intermediates, blackjacks and yellow pines, and intermediates and yellow pines. Graphic representations of the tests are given in figures 5, 6, and 7, in which

[12] MINER, J. R. THE STANDARD ERROR OF A MULTIPLE REGRESSION EQUATION. Ann. Math. Statis 2: 320-323. 1931.

[13] SCHUMACHER, F. X., and HALL, F. DOS S. See footnote 8.

[14] Roughly, the point at which the odds are 1 to 20 that the two samples came from the same universe.

each small cross (+) indicates the pairs of values of X_2, X_3 for which volumes were computed by each equation. The number near each cross is the ratio of the difference in volume, computed by each equation, to the standard error of the difference. A ratio of 1.9599 or larger is considered significant. It should be noted that the area

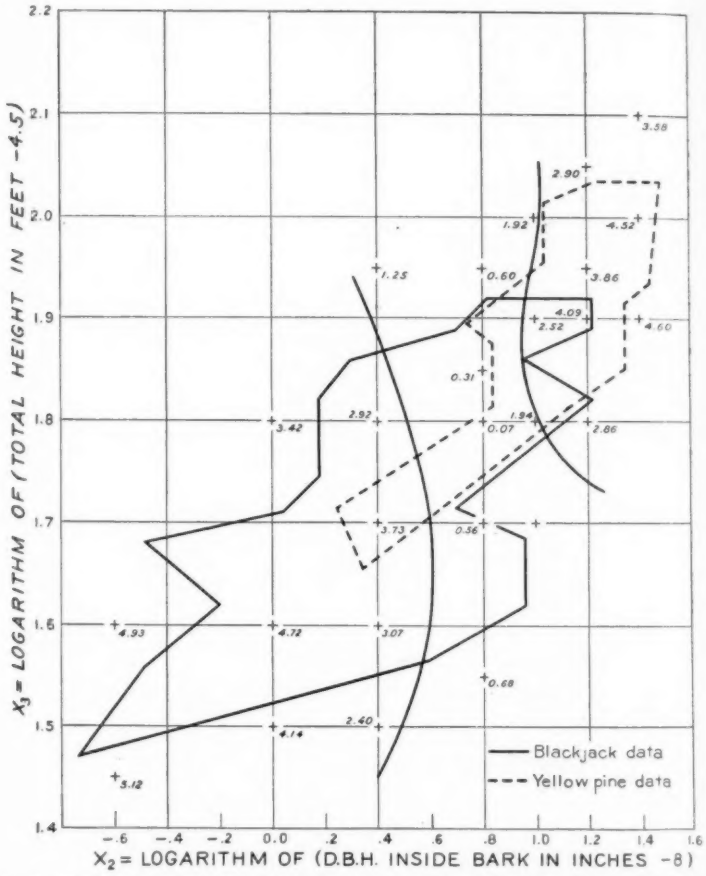

FIGURE 6.—Graphical representation of the test for determining the significance of the difference between the volume equation of the yellow pine group and of the blackjack group. The volumes, as estimated by the two equations, are significantly different excepting in the region of X_2, X_3, lying between the two contours.

between the contours of two standard errors of the difference represents the distribution of logarithms of (diameter breast high −8) and (height −4.5) in which there is no significance or where either volume equation could be used satisfactorily.

Since there was no significant difference between the equations for blackjacks and intermediates, these groups were combined and

compared with yellow pines. Figure 8 is a graphic representation of this test and shows that the volume equations for blackjacks and intermediates combined is significantly different from that of yellow pines throughout the major part of their range of diameters and heights. Applying the data used in this test to figure 8, it was found

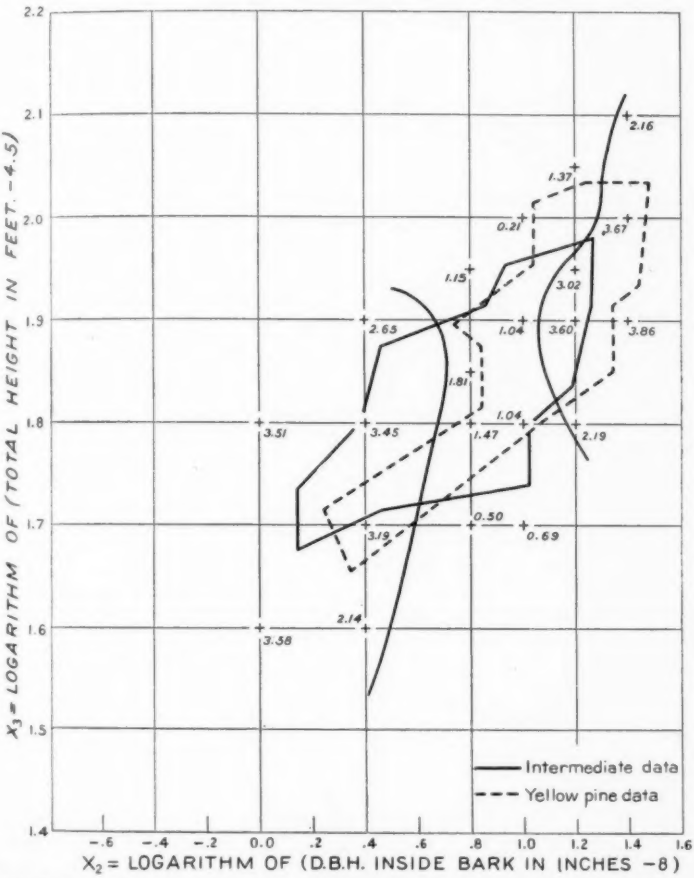

FIGURE 7.—Graphical representation of the test for determining the significance of the difference between the volume equation of the yellow pine group and of the intermediate group. The volumes, as estimated by the two equations, are significantly different excepting in the region of X_2, X_3 lying between the two contours.

that 10.9 percent of the yellow pines and 43.4 percent of the blackjacks and intermediates combined fell within the region of no significance or where either equation could be used satisfactorily. This means, so far as these data are concerned, that two volume tables should be constructed, one for yellow pines and one for the combined group of blackjacks and intermediates.

ALINEMENT-CHART VOLUME TABLES

The alinement-chart form of volume table is especially valuable for use in growth or increment studies, since it makes it possible to use diameter measurements to the nearest one-tenth inch and height

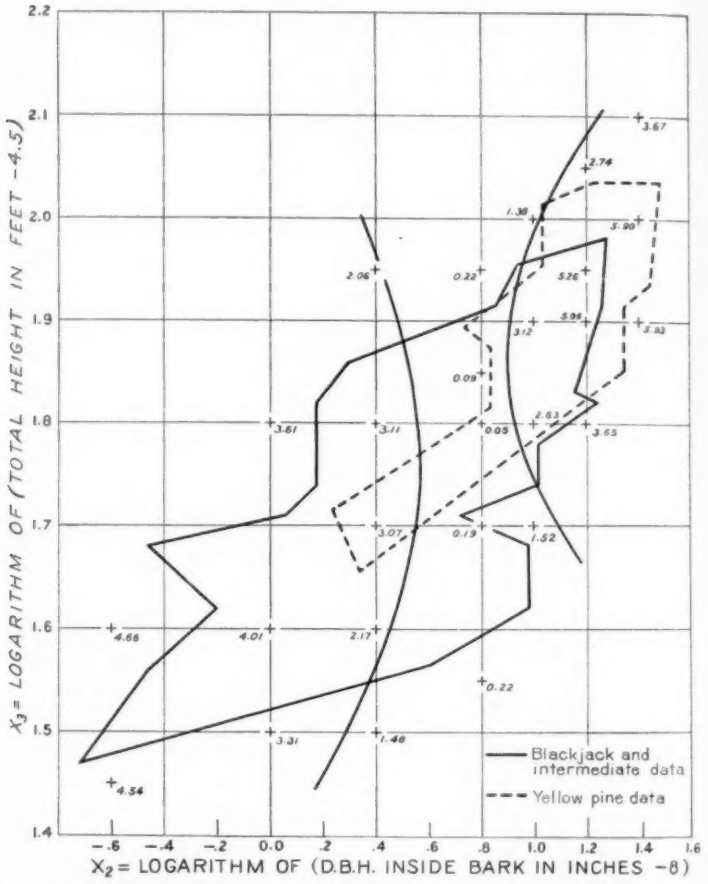

FIGURE 8.—Graphical representation of the test for determining the significance of the difference between the volume equation of the yellow pine group and of the combined blackjack-intermediate group. The volumes, as computed by the two equations, are significantly different excepting in the region of X_2, X_3 lying between the two contours.

measurements to the nearest foot and to determine volumes of small and large trees with the same relative degree of accuracy.

The logarithmic form of the volume equations which have been presented is readily expressed in alinement-chart form. Three straight parallel axes for log (diameter breast high −8), log (volume −6.6), and log (height −4.5), respectively, are used, upon which the logarithmic functions of the variables are graduated in the arith-

metic scale.[15] Corrections are made for constants that were subtracted from the variables before these were entered into logarithms, i. e., to volume is added 6.6 board feet, to diameter breast high inside bark is added 8 inches and double bark thickness, and to

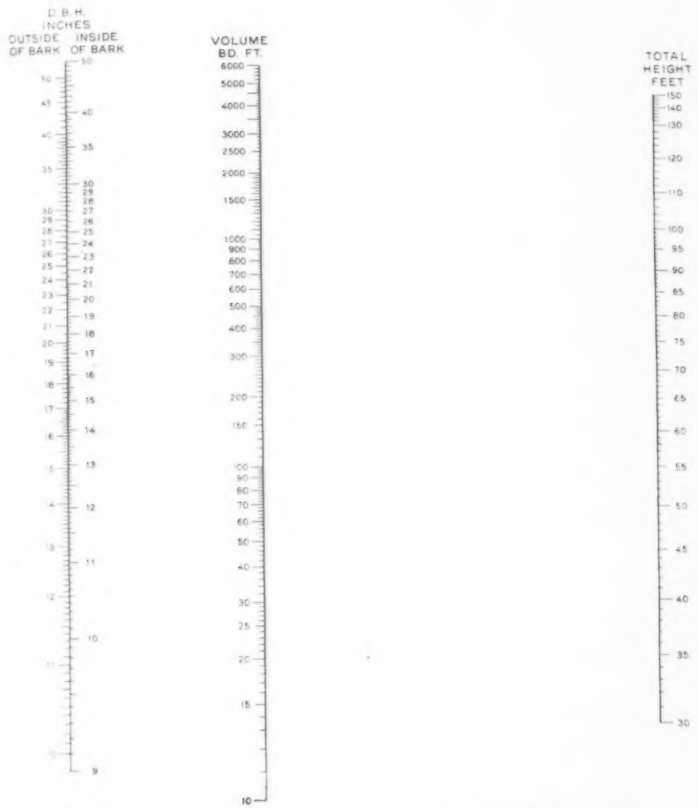

FIGURE 9.—Alinement-chart volume table for mature ponderosa pine (yellow pine).

height is added 4.5 feet. Figures 9 and 10 represent the volume tables in their final alinement-chart form. Tables 3 and 4 give the data in tabular form.

[15] BRUCE, D., and REINEKE, L. H. CORRELATION ALINEMENT CHARTS IN FOREST RESEARCH: A METHOD OF SOLVING PROBLEMS IN CURVILINEAR MULTIPLE CORRELATION. U. S. Dept. Agr. Tech. Bull. 210, 88 pp., illus. 1931.

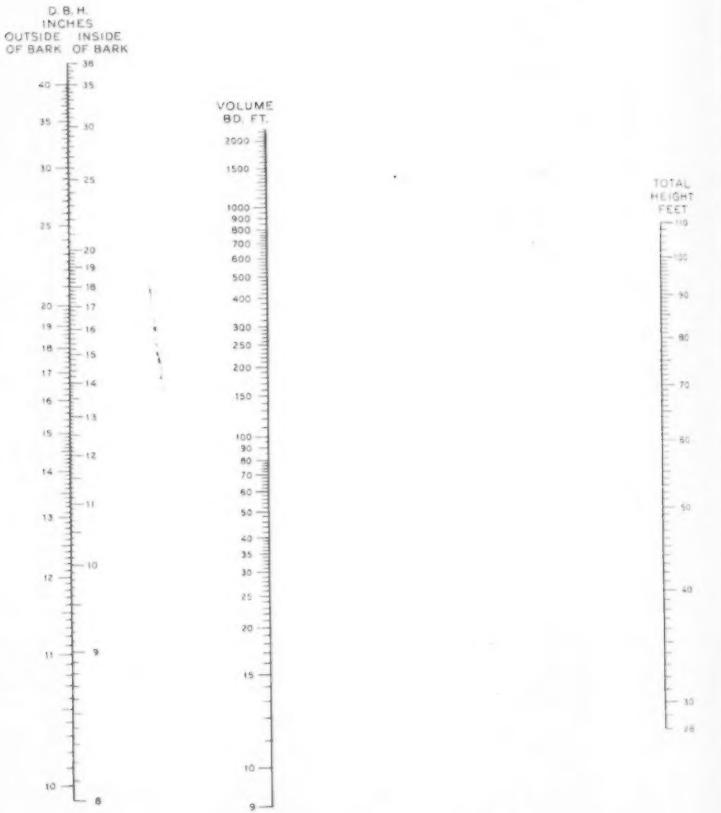

FIGURE 10.—Alinement-chart volume table for immature ponderosa pine (blackjacks and intermediates).

TABLE 3.—*Board-foot volume table, Scribner rule, for yellow pine (mature ponderosa pine)* [1]

Diameter breast high		Volume when the total height of tree is—									Basis (trees)
Outside bark (inches)	Inside bark (inches)	40 feet	50 feet	60 feet	70 feet	80 feet	90 feet	100 feet	110 feet	120 feet	
		Board feet	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Number*
10	9.10	15	18	22	25						0
11	9.75	22	28	34	40						1
12	10.50	31	40	50	60	71					0
13	11.25	41	54	68	82	98					2
14	12.08	53	70	89	108	128	149				0
15	12.90	66	88	112	137	163	190	218			2
16	13.80	80	108	137	169	203	235	270			2
17	14.70	96	129	166	204	244	284	327	370		2
18	15.65	113	153	197	240	288	337	387	440	490	5
19	16.60	130	177	227	279	334	391	448	508	575	7
20	17.50	147	201	258	317	382	444	514	582	660	5
21	18.43		225	290	359	430	498	578	656	740	9
22	19.35		251	322	399	479	560	645	730	825	10
23	20.25		278	360	440	530	622	717	810	915	10
24	21.30		306	397	488	590	690	790	895	1,015	10
25	22.30		339	435	537	645	755	870	984	1,110	15
26	23.25		369	473	585	707	821	946	1,075	1,210	13
27	24.15		398	514	635	760	890	1,025	1,164	1,300	12
28	25.10		430	558	690	825	964	1,110	1,255	1,420	14
29	26.10			603	740	890	1,040	1,200	1,352	1,525	14
30	27.10			644	787	950	1,107	1,277	1,445	1,630	5
31	28.00			688	840	1,016	1,185	1,360	1,540	1,744	11
32	29.00			727	895	1,080	1,255	1,445	1,640	1,858	7
33	30.00			770	947	1,145	1,333	1,530	1,740	1,970	5
34	31.00			820	1,000	1,210	1,409	1,624	1,846	2,100	1
35	31.95				1,057	1,275	1,480	1,710	1,950	2,210	2
36	32.90				1,112	1,345	1,558	1,800	2,060	2,320	4
37	33.90				1,168	1,400	1,640	1,890	2,164	2,425	2
38	34.80				1,225	1,465	1,720	1,990	2,270	2,520	1
39	35.75				1,270	1,530	1,790	2,090	2,370	2,650	2
40	36.80				1,345	1,610	1,880	2,193	2,480	2,790	0
41	37.80					1,694	1,989	2,300	2,600	2,910	0
42	38.80					1,760	2,080	2,400	2,710	3,050	0
43	39.81					1,850	2,180	2,500	2,840	3,200	1
44	40.90					1,930	2,275	2,600	2,952	3,350	0
Basis (trees)		0	3	2	19	63	56	22	9	0	174

[1] Block indicates extent of basic data. Prepared by the method of least squares. Basis: 174 trees measured by the Southwestern Forest and Range Experiment Station on the Sitgreaves National Forest, Ariz. Malpais area: Average height of mature dominant trees, 95 feet; stump height, 1 foot; top diameter inside bark, 8 inches; scaled in 16-foot log lengths with trimming allowance of 0.3 foot, additional top sections scaled as fractions of 16-foot, 8-inch log. Aggregate deviation, chart 0.43 percent low, average percentage deviation trees 10 inches +, 8.5 percent; values read from alinement chart.

In using these volume tables, discretion is necessary. It should be remembered that they will give results within the standard error specified only if they are applied to trees growing in stands similar to those from which the data were taken. It does not appear, however, that this limitation will greatly restrict the use of the volume tables on stands cut under present Forest Service cutting practice.

TABLE 4.—*Board-foot volume table, Scribner rule, for blackjacks and intermediates (immature ponderosa pines)*

Diameter breast high		Volume when the total height of tree is—									Basis (trees)
Outside bark (inches)	Inside bark (inches)	30 feet	40 feet	50 feet	60 feet	70 feet	80 feet	90 feet	100 feet	110 feet	
		Board feet	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Board feet*	*Number*
10	8.07	10	12	14	15						4
11	8.97	15	19	23	28	32					11
12	9.83	21	28	35	42	49					11
13	10.75	29	39	49	60	71	82				21
14	11.64	37	50	65	80	95	111				10
15	12.55	46	64	82	102	122	143				24
16	13.45	55	78	101	127	152	177				15
17	14.33		92	122	152	182	213	248			22
18	15.23		109	144	179	215	252	291			16
19	16.13		126	167	207	250	292	338			19
20	17.04			190	239	286	335	387	440	496	20
21	18.00			216	272	326	381	441	504	563	10
22	18.85			242	303	364	428	497	561	633	19
23	19.78			270	336	405	476	552	625	702	6
24	20.60			296	370	446	523	609	688	770	10
25	21.55			324	408	492	578	667	758	847	7
26	22.47			354	442	536	628	724	824	925	1
27	23.30				482	582	682	785	892	998	3
28	24.22				520	627	733	847	955	1,078	1
29	25.07				560	673	785	910	1,040	1,155	3
30	26.00					726	850	980	1,116	1,255	3
31	26.90					775	910	1,055	1,200	1,350	1
32	27.85					830	970	1,122	1,280	1,448	0
33	28.75					885	1,035	1,200	1,365	1,540	0
34	29.60						1,095	1,265	1,450	1,630	0
35	30.50						1,154	1,349	1,535	1,730	0
Basis (trees)		2	4	31	50	70	50	27	3	0	237

[1] Block indicates extent of basic data. Prepared by the method of least squares. Basis: 237 trees measured by the Southwestern Forest and Range Experiment Station on the Sitgreaves National Forest, Ariz. Malpais area: Average height of mature dominant trees, 95 feet; stump height, 1 foot; top diameter inside bark, 8 inches; scaled in 16-foot log lengths with trimming allowance of 0.3 foot, additional top sections scaled as fractions of 16-foot, 8-inch log; aggregate deviation, chart 0.12 percent high; average percentage deviation trees 12 inches +, 13.0 percent; values read from alinement chart.

SUMMARY AND CONCLUSIONS

This paper presents the methods and findings in the development of Scribner board-foot volume tables for cut-over stands of ponderosa pine on the Malpais soil type in Arizona. Improved methods of comparing the volume of the three age classes of ponderosa pine (blackjacks, intermediates, and yellow pines) are described. The methods used, which follow the technique developed by Schumacher and Hall, represent a radical departure from the methods heretofore used in constructing volume tables.

The tables are presented in alinement-chart and tabular form.

For the Scribner board-measure rule and for the data used in this study, the following conclusions may be drawn:

(1) The volume equation for blackjacks does not give a result significantly different from that for intermediates.

(2) The volume equation for yellow pines does give a result significantly different from that for blackjacks or from that for intermediates.

(3) The volume equation for yellow pines gives a result significantly different from that for blackjacks and intermediates combined.

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